TABLE OF CONTENTS

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1964-66	B.Hetzel	V.Trikojus	I.Jarrett	I.Jarrett
1966-68	B.Hudson	V.Trikojus	R.Melick	I.Jarrett
1968-70	P.Taft	R.Cox	R.Melick	I.Jarrett
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1972-74	K.Ferguson	L.Lazarus	R.D.Gordon	L.Lazarus
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1978-80	J.P.Coghlan	C.J.Eastman	R.G.Larkins	J.W.Funder
1980-82	C.J.Eastman	J.W.Funder	D.P.Cameron	G.L.Warne
1982-84	J.W.Funder	R.G.Larkins	R.C.Baxter	G.L.Warne
1984-86	R.G.Larkins	D.P.Cameron	R.C.Baxter	D.M.Hurley
1986-88	D.P.Cameron	R.C.Baxter	S.J.Judd	D.M.Hurley
1988-90	R.C.Baxter	S.J.Judd	J.R.Stockigt	D.J.Handelsman
1990-92	J.R.Stockigt	J.A.Eisman	G.W.Tregear	D.J.Handelsman
1992-94	D.J.Handelsman	P.J.Fuller	R.L.Prince	D.J.Topliss
1994-96	P.J.Fuller	R.L.Prince	G.P.Risbridger	D.J.Topliss
1996-98	D.J.Topliss	R.J.Rodgers	G.P.Risbridger	M.S.Lewitt
1998-00	R.J.Rodgers	J.D.Zajac	K.K.Y.Ho	M.S.Lewitt
				B.J.Waddell (May 99)
2000-02	K.K.Y.Ho	B.J.Waddell	B.Canny	C.Coulter
2002-03	B.Canny	J.D.Zajac	R. Cuneo	C.Coulter

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2002	George Chrousos
2003	Derek LeRoith
2004	Bruce McEwen

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The Novartis Junior Award is awarded annually to a member who is a postgraduate student or recent post-doctoral student, for the best original paper at the Annual Scientific Meeting.

1976	Kathryn Rich & Peter Fuller
1977	David Kennaway
1978	David Healy
1979	George Werther
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1981	Yvonne Hodgson
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1992	Fiona Young
1993	Emma Ball
1994	Vicki Clifton
1995	Michael Downes & Sylvia Lim-Tio
1996	John Walsh
1997	Bu Yeap
1998	Julie Joyner
1999	Renea Jarred & Helena Teede
2000	Jeremy Smith
2001	Stephen Heady
2002	Patrick McManamny
2003	Sophie Chan

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The Servier Award is awarded for the best published work in the previous year by a member of the Society within 5 years of award of higher degree.

1991	Sharon Gargosky
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1994	Chen Chen
1995	Timothy Crowe
1996	Jun-Ping Lui
1997	Liza O'Donnell
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2000	Fraser Rogerson
2001	Karen Kroeger
2002	Susan Fanayan
2003	Jenny Gunton

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SOCIETY SECRETARIAT
Endocrine Society of Australia (ESA)
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PRINCIPAL GUEST SPEAKERS FOR 2004

Professor R. John Aitken (Australia)
Professor R. John Aitken is currently Director of the ARC Centre of Excellence in Biotechnology and Development and Head of Biological Sciences at the University of Newcastle. He is a Fellow of the Royal Society of Edinburgh and has published more than 400 articles in the general area of reproductive biology with emphasis on the cell biology of male germ cells, contraception and infertility

Professor Peter Clayton (USA)
Professor Clayton graduated from Manchester University Medical School in 1984 (Distinction in Paediatrics), having obtained a first class degree in Physiology & Pharmacology in 1981. He did his early paediatric training around Manchester before embarking on an academic career in Paediatric Endocrinology. His MD thesis was on "Growth Patterns after Neuroaxis Irradiation in Childhood". He spent time at the University of Virginia, USA as a MRC Travelling Fellow in 1990/1 with his work there primarily directed at molecular endocrinology in the laboratory.
He returned to Manchester to establish a research group of both clinicians and scientists, working under the broad remit of investigating normal and abnormal growth from clinical, biochemical and molecular perspectives. He took up a Senior Lectureship in Child Health in 1994, and was promoted to Professor of Child Health and Paediatric Endocrinology in 2001. He is presently leader of the Human Development & Reproductive Health Clinical Academic Group at the University of Manchester.

Professor Aaron J.W. Hsueh (USA)
Aaron Hsueh is a Professor at Stanford University School of Medicine. He has investigated gonadotropin and paracrine regulation of granulosa cell differentiation. The Hsueh lab launched the Ovarian Kaleidoscope database to document ovarian genes. He recently pioneered the use of bioinformatic approaches leading to the discovery of novel polypeptide hormones and receptors. Dr. Hsueh and his colleagues identified multiple leucine-rich repeat-containing, G protein-coupled receptors (LGRs), two of them as receptors for the long sought relaxin and INSL3 receptors. Based on evolutionary genomic analyses, his lab launched the Plasma Membrane Receptome (HPMR) database that documents >1,000 plasma membrane receptors.

Professor Bruce S. McEwen (USA)
Bruce McEwen, Ph.D., is the Alfred E. Mirsky Professor and Head of the Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology at The Rockefeller University. McEwen graduated Summa Cum Laude in Chemistry from Oberlin College in 1959 and obtained his Ph.D. in Cell Biology in 1964 from The Rockefeller University. He returned to Rockefeller in 1966 to work with the psychologist, Prof. Neal Miller, after postdoctoral studies in neuobiology in Sweden and a brief period on the faculty at the University of Minnesota. He was appointed as Professor at Rockefeller in 1981. He is a member of the US National Academy of Sciences, the Institute of Medicine, the American Academy of Arts and Sciences and a Fellow of the New York Academy of Sciences. He served as Dean of Graduate Studies from 1991-3 and as President of the Society for Neuroscience in 1997-98.

As a neuroscientist and neuroendocrinologist, McEwen studies environmentally-regulated, variable gene expression in brain mediated by circulating steroid hormones and endogenous neurotransmitters in relation to brain sexual differentiation and the actions of sex, stress and thyroid hormones on the adult brain. His laboratory discovered adrenal steroid receptors in the hippocampus in 1968. His laboratory combines molecular, anatomical, pharmacological, physiological and behavioral methodologies and relates their findings to human clinical information. He is a member of the MacArthur Foundation Research Network on Socioeconomic Status and Health, in which he is helping to reformulate concepts and measurements related to stress and stress hormones in the context of human societies. He is the co-author of a new book with science writer Elizabeth Lasley for a lay audience called "The End of Stress as We Know It" published by the Joseph Henry Press and the Dana Press.

Professor Hironobu Sasano (Japan)
Professor Sasano is Head of the Department of Pathology, Tohoku University School of Medicine and Director of the Department of Pathology, Tohoku University Hospital. After graduating in 1986 from Tohoku University, he spent several years at George Washington University in the Department of Pathology. He has since returned to take up his current position at Tohoku University and continue his research interests in Steroid biosynthesis, metabolism and actions.

Philipp E. Scherer (USA) – ADS Speaker
The main focus in Dr Scherer's laboratory is the identification and physiological characterization of adipocyte-specific gene products and the elucidation of pathways that are an integral part of the complex set of reactions that drive adipogenesis. Their hope is to unravel novel mechanisms and identify novel proteins that could serve as potential links between the adipocyte and the process of whole body energy homeostasis. It is now becoming clear that adipocytes are highly responsive to extracellular stimuli, play a central role in overall energy homeostasis and are also essential for certain aspects of the immune system. Other than leptin and adipsin, Adiponectin is the only other secretory protein described to date that is predominantly released from adipocytes. Dr Scherer's group described the initial cell biological characterization of Adiponectin. They have cloned the human homolog of Adiponectin and started to study Adiponectin expression levels in patients. In agreement with their tissue culture data, they have recently published data showing that elevated levels of insulin lead to an acute increase in serum Adiponectin levels; chronically elevated levels of insulin lead to decreased serum insulin levels in patients. They have also been involved in studies showing that muscle and liver are the primary target organs for Adiponectin.

Professor Tom Spencer (USA)
Thomas E. Spencer (b. 1968) earned his B.S. and M.S. in Animal Science at Auburn University and Ph.D. in Reproductive Biology from Texas A&M University. He was a NIH Postdoctoral Fellow in Cell and Molecular Biology at Baylor College of Medicine from 1995 to 1997. He is currently an Assistant Professor of Reproductive Biology and Physiological Genomics in Animal Science. His research focuses on the hormonal, cellular and molecular mechanisms governing development and function of the uterus using sheep as a model system. He currently serves as a member of the Editorial Boards for the journals of Biology of Reproduction, Domestic Animal Endocrinology, and Reproduction.

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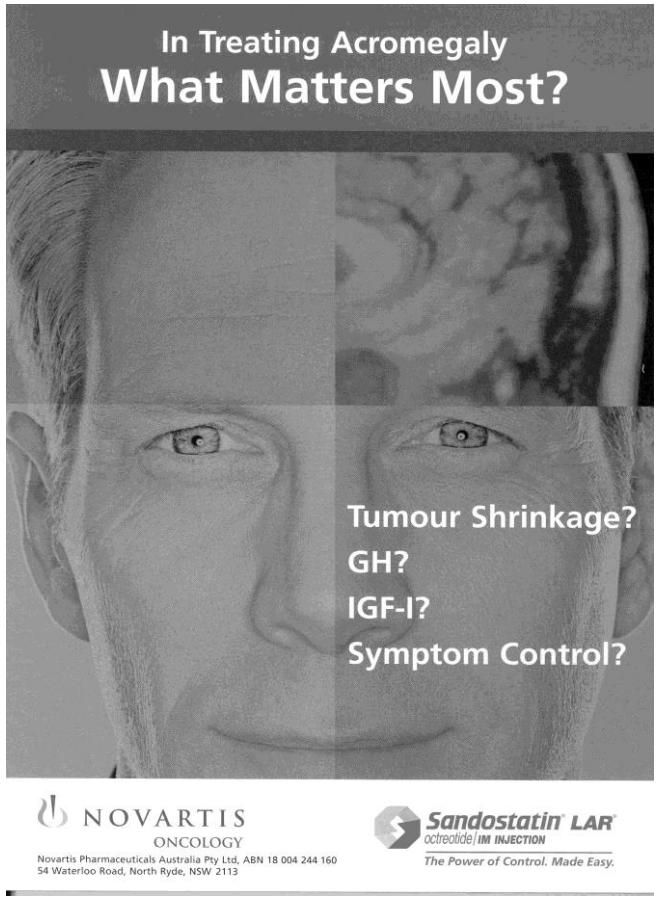
At Novartis Oncology, the pursuit for excellence in research, clinical trial development and local initiatives is the commitment we make to health care providers and patients. The Novartis representatives present at this meeting would be happy to answer any questions related to Novartis Oncology products.



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The organiser's office and registration desk will be located on the first level in the foyer area of the Sydney Convention Centre. It is accessible by the escalators from the main foyer entrance. The office and desk will be attended at all times during the conference from 7:30am in the morning. Delegates should collect their satchel, name tag and other conference material on arrival. A message board will be placed immediately inside the main Exhibition entrance.

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Conference delegates receive the following services as part of their registration:

- Access to all scientific sessions on day(s) of registration
- A satchel with a copy of the delegate handbook and abstracts*
- Lunches on Monday, Tuesday and Wednesday
- Morning teas on Monday, Tuesday and Wednesday
- Afternoon teas on Monday and Tuesday
- The Welcome Function on Sunday evening

*All delegates receive a copy of the proceedings, but satchels can only be given to trade delegates if supply allows

Name Tags
Delegates are required to wear their name tags to all scientific and catered sessions.

Session Locations
The Conference activities are spread out over three levels. The Bayside Banquet Hall, located on the ground level, is where the exhibition is and all breaks are taken there. The scientific sessions are generally located on level two. All rooms on this level are "Harbourside" rooms. The next level rooms are called "Skyline" rooms. All these rooms are accessed by escalators. The posters are on the Harbourside level in the foyer.

The Speaker Preparation Room

The speaker preparation room is Harbourside Meeting Room 8. Networked computers in this room will allow MS PowerPoint presentations loaded here to be shown in any of the session rooms. Technicians and assistants will be in attendance in the room and speakers are encouraged to load their presentations as soon as possible to avoid any last minute rushes. Should delegates be using 35mm slides, they must inform the secretariat as early as possible to ensure the equipment is booked for their session as these projectors are not standard in each room. Carousels will be available in the preparation room. Those using slides should collect them immediately at the end of their session. The organisers are not responsible for slides not claimed at the end of the session.

Poster Viewing

Delegates with posters can find the correct position for their poster by finding the appropriate abstract number on the display panels in the Harbourside foyer. The program provides your abstract number which is how you find you placement position. Posters can remain on display all of Monday and Tuesday and must be removed after the days sessions on Tuesday. During formal poster discussions, the presenters should be present at their poster to answer questions and meet colleagues with similar research interests.

Social Functions

- The **Welcome Function** is in the Convention Centre in the Harbourside Room on the first level (located on the same level as the registration desk, up the escalators) on the Sunday evening from 6pm. Light refreshments and drinks will be served and the function is complimentary for all registration types.
- The **Women in Endocrinology Function** will follow the Welcome Function at 7pm and is in Skyline 1 (take the escalators to level three). Again light refreshments and drinks will be served. This is a ticketed function and they must be purchased in advance.
- The Monday night **Student Function** is at The Blackbird Café - Balcony Level, Cockle Bay Wharf, Darling Harbour (follow the walkway from the Convention Centre around to the Cockle Bay Wharf precinct). Maps will be at the registration desk for those not sure how to find it. Those who have already purchased a ticket should find their ticket with their registration papers on arrival. The ticket cost includes your meal, entertainment and drinks for the first two hours. The function begins at 7:30pm and dress is neat casual. This is a ticketed function and they must be purchased before the night.
- The **Conference Dinner** will be held onsite at the Convention Centre in the Harbourside Room (located on the same level as the registration desk, up the escalators). Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. Entertainment for the night is provided by 'NightStand'. This is a ticketed function and they must be purchased in advance.

Insurance

The hosts and organisers are not responsible for personal accidents, any travel costs, or the loss of private property and will not be liable for any claims. Delegates requiring insurance should make their own arrangements.

Disclaimer

The hosts, organisers and participating societies are not responsible for, or represented by, the opinions expressed by participants in either the sessions or their written abstracts.

Smoking - is not permitted in the venue.

Mobile Phones - Please ensure they are turned off during any session you attend.

Message Board - will be available at the registration desk on the first floor foyer.

Occasional Meetings

A number of special meetings and functions have been called by various interested parties throughout the conference. Those involved and uncertain of which room they should be in will be able to obtain guidance from the registration desk.

The Trade Passbook Competition

Amongst delegate's registration papers is a "Trade Pass Book" entry form. The form has spaces for the stamp or signature of each of the trade exhibitors. Forms are completed by visiting all of the trade sites, meeting their representatives and registering their 'stamp' in the designated space. Once you have collected 15 stamps or signatures, place your completed form in the entry box at the registration desk by the end of afternoon tea on the Tuesday. The prize for the first completed entry form, drawn at the dinner is donated by ASN. *Trade representatives are not eligible to enter the competition.

FUTURE MEETINGS

2004

SRB Symposium Day
As part of the 2nd Australian Health & Medical Research Congress
22nd - 26th November 2004
Sydney Convention Centre North, NSW
www.esa-srb.org.au

2005

ESA Seminar 2005
15th – 17th April 2005
venue in Victoria to be confirmed
www.esaseminar.org.au

ESA Clinical Weekend 2005
2nd – 4th September 2005
The Vines Novotel, Swan Valley WA
www.esaclinicalweekend.org.au

Combined ESA/SRB Annual Scientific Meeting
4th – 7th September 2005
Perth Convention Centre, WA
www.esa-srb.org.au

Insert exhibition floor plan

Sunday, 22 August 2004

Session 1 - SRB Symposium: Molecular Regulation of Fertilisation and Early Embryonic Development

4:00 PM - 6:00 PM Harbourside Rm 3

Session sponsored by University of Adelaide Centre for Reproductive Health

Chairs: Rob Norman and Peter Kaye

4:00 **John Carroll**
Coordinating the transition from egg to embryo in mammals *abs#010*

4:30 **Keith Jones**
Waking up the egg. How the sperm regulates exit out of the meiotic cell cycle *abs#011*

5:00 **Jeremy Thompson**
Metabolic determinants of implantation success and programming long term viability in embryos *abs#012*

5:30 **Chris O'Neill**
The regulation of survival of the pre-implantation embryo *abs#013*

ESA/SRB Welcome Function

6:00 PM - 7:30 PM Harbourside Function Room

Women in Endocrinology Function

7:00 PM - 8:30 PM Skyline 1

Function sponsored by DSL Labs

Monday, 23 August 2004

Session 2 - SRB orals #1 - Oocyte/Follicle

8:30 AM - 10:00 AM Harbourside Rm 3

Chairs: Kara Britt and John Carroll

8:30 **Lesley Ritter**
Regulation of mouse mural granulosa cell progesterone synthesis by oocyte paracrine factors *abs#201*

8:40 **Kara Britt**
Estrogen actions on follicle formation and early follicle development *abs#202*

8:50 **Bridget Sisco**
Studies of the Activin pathway in dominant and subordinate bovine follicles before, during and after selection *abs#203*

9:00 **Melanie Sutton-McDowall**
Regulation of bovine oocyte meiotic and developmental capacity by glucose and glucosamine *abs#204*

9:10 **Katherine Morton**
The effect of FSH concentration during IVM and gamete co-incubation length during IVF on the development of unstimulated prepubertal ewe oocytes *abs#205*

9:20 **Raymond Rodgers**
Cell Death of the Theca Interna during Bovin Ovarian Follicular Atresia *abs#206*

9:30 **Jonathan Paul**
Oolemmal proteomics: Identification of Oocyte Cell Surface Protein Complexes involved in Sperm-Egg Interaction *abs#207*

9:40 **Janine Duckworth**
Zona pellucida vaccines for fertility control of brushtail possums in New Zealand *abs#208*

Session 3 - SRB orals #2 - Spermatogenesis

8:30 AM - 10:00 AM Harbourside Rm 4

Chairs: Eileen McLaughlin and Stephan Schlatt

8:30 **Mark Baker**
A proteomic analysis of rat caput and cauda sperm using using difference in 2D-gel electrophoresis *abs#209*

8:40 **Cathryn Hogarth**
Regulated expression of mRNAs encoding nuclear transport proteins during spermatogenesis *abs#210*

8:50 **Camden Lo**
Subcellular localization of testis forms of MAP2 is a product of competing targeting signals/domains *abs#211*

9:00 **Brett Nixon**
The molecular basis of epididymal sperm maturation *abs#212*

9:10 **Catherine Itman**
Culture of Mouse Male Germ Cells for Genetic Manipulations *abs#213*

9:20 **Andrew Walsh**
Identification of potential Zona Pellucida-binding sperm proteins using heat shock protein 60 *abs#214*

9:30 **Liga Bennetts**
Analysis of DNA Damage Induced by Pro-Oxidant Treatment of Mammalian Spermatozoa In Vitro *abs#215*

9:40 **Sheena Lewis**
The effects of Viagra on Sperm Function and Early Embryo Development *abs#216*

Session 4 - ESA Australia-Japan Lecture

8:45 AM - 9:45 AM Harbourside Auditorium

Chair: Stephen Twigg

Hironobu Sasano
Intracrinology of human breast cancer – intratumoral estrogen production *abs#001*

Session 5 - ESA Servier Award

9:45 AM - 10:00 AM Harbourside Auditorium

Session sponsored by Servier

Peter Liu
The Short-term Effects of High Dose Testosterone on Sleep, Breathing and Function in Older Men *abs#044*

Morning Tea

10:00 AM - 10:30 AM Exhibition Area

Break sponsored by GlaxoSmithKline

Session 6 - ESA Novartis Junior Investigator

10:30 AM - 12:00 PM Harbourside Auditorium

Session sponsored by Novartis

Chair: Stephen Twigg and Ben Canny

10:30 **Esme Hatchell**
Cloning Of A Novel Sra-binding Protein, SLIRP, That Regulates Estrogen Action in Cancer Cells *abs#101*

10:45 **Morton Burt**
Impact of Chronic Low Dose Glucocorticoids on Body Composition and Protein Metabolism *abs#102*

11:00 **Andrew Redfern**
The role Of Novel Sra-binding Proteins in Nuclear Receptor Pathway co-regulation *abs#103*

11:15 **Rachel Hill**
Lack of Estrogen In Male Mice May Be Linked To Obsessive Compulsive Disorder *abs#104*

11:30 **Daniel Scott**
LGR7-truncate Is an Alternative mRNA Splicing Variant of The Relaxin Receptor, LGR7, And Is A Relaxin Antagonist in Vitro *abs#105*

11:45 **Katie Dixon**
Mechanism of Photoprotection by Vitamin D Compounds *abs#106*

Session 7 - SRB Symposium: The Uterine / Placental Interface: New Genes, New Dimensions

10:30 AM - 12:00 PM Harbourside Rm 3

Chairs: Claire Roberts and Rebecca Jones

10:30 **Guiying Nie**
Novel uterine genes in regulation of embryo implantation *abs#014*

11:00 **Lorraine Robb**
Disrupted decidualization in SOCS3 gene mutant mice *abs#015*

11:30 **Sally Dunwoodie**
Role of Cited genes in placental morphogenesis: studies in null mutant mice *abs#016*

Session 8 - SRB Founders Lecture

12:00 PM - 1:00 PM Harbourside Auditorium

Session sponsored by Reproduction, Fertility and Development

Chair: Lois Salamonsen

Robert Aitken
Human spermatozoa: fruits of creation, seeds of doubt *abs#002*

Lunch

1:00 PM - 2:00 PM Exhibition Area

Session 9 - ESA Posters #1

2:00 PM - 3:00 PM ***posters can be displayed all of Monday and Tuesday*** Harbourside Foyer

Elizabeth Rivalland
Distribution and co-expression of enkephalin and peptides of the stress axis in the paraventricular nucleus of male and female sheep *abs#302*

Kristy Shipman
Identification of a DNA binding protein family with similarity to RNA splicing factors *abs#303*

Warwick Bagg
Despite differences in body composition IGF-I levels are similar in Samoan, Maori and European populations *abs#304*

David Torpy
The First International Randomized Trial in Locally Advanced and Metastatic Adrenocortical Carcinoma Treatment (FIRM-ACT) *abs#305*

Niroshani Pathirage
Aromatase is not expressed in endometrial cancer *abs#306*

Rudi Allan
Novobiocin inhibits cross-talk between heat shock protein 90 dimerisation and ATP-binding domains *abs#307*

Helen Irving-Rodgers
Composition of the murine pancreatic islet basal lamina *abs#308*

Mathis Grossmann
ACTH-independent macronodular adrenal hyperplasia: A rare cause of cushing's syndrome *abs#309*

Mark McLean
Australian and New Zealand acromegaly registry (ANZAR): Progress in development of a collaborative resource *abs#310*

Kerry-Lee Milner
Cushing's syndrome as a manifestation of carney complex: a case report *abs#311*

Indra Jayasuriya
Challenges in the Management of Adrenocorticotropin Releasing Hormone [ACTH] Secreting Pituitary Macroadenomas *abs#312*

Stephen Twigg
A Case of Metformin-induced Hypoglycemia *abs#313*

Ildi Koves
The Mystery of the parathyroid: A case report of a twin pregnancy with paradoxical maternal and infant outcomes *abs#314*

Carolyn Mitchell
Solid phase extraction of prostaglandins E_2, $F_{2\alpha}$ and their metabolites from biological samples *abs#316*

Kym Rae
Follistatin Changes Significantly in Spontaneous Labouring Women *abs#317*

Sean Yang
Regulation of voltage-gated K^+ currents of rat somatotropes by somatostatin receptors *abs#318*

Maria Hernandez
Time-dependent effects of GHRH and GHRP-2 on the expression rates of GH, GHRH-receptor, GHS-receptor, somatostatin receptors (sst) and Pit-1 and the secretion of GH in primary cultured ovine pituitary cells *abs#319*

Brian Oldfield
The Role Of Thermogenesis In The Resistance To Diet Induced Obesity *abs#320*

Bi-ke Zhu
Expression of mRNA for hormones regulating feeding responses in the neonatal pig stomach *abs#321*

Timothy Cole
Glucocorticoid receptor expression from the 1A promoter correlates with T lymphocyte sensitivity to glucocorticoid-induced cell death *abs#322*

Shane Patella
Activin A and follistatin response in acute hepatic inflammation *abs#323*

Daniel Bird
Expression of Type I & Type II Alveolar Epithelial cell Marker Genes in the Developing Lung of GR-/- Mice *abs#324*

Denise Goldman
Characterisation of the androgen receptor pathway in early embryonic development *abs#325*

David MacIntyre
Western Blot Characterisation Of The Human Myometrium In Labour *abs#326*

Hannah Palliser
Prostaglandin F and E Synthase and Receptor Expression in the Amnion and Cervix of the Parturient Ewe *abs#327*

Michael Epis
Hur Interactions With The 3'-UTR of EGF Receptor (EGF-R-r) and C-*erb*-b2 (her-2) in Human Breast Cancer *abs#328*

Mathis Grossmann
A four year audit of severe hypotonic hyponatraemia at a general hospital *abs#329*

Miles De Blasio
Maternal exposure to dexamethasone or cortisol in early pregnancy causes fasting hyperglycaemia and hyperinsulinaemia in adult male offspring in sheep *abs#330*

Session 10 - SRB orals #3 - Ovary / CL

2:00 PM - 3:30 PM Harbourside Rm 3

Chairs: Ann Drummond and Arun Dharmarajan

2:00 **Janet Holt**
Expression of the chemokine CXCl12 and its receptor CXCR4 in the activating mammalian ovarian follicle *abs#217*

2:12 **Ann Drummond**
FGF9 stimulates ovarian progesterone production *abs#218*

2:24 **Cadence Haynes**
Analysis of Ovarian Macrophage Populations using Macrophage-Specific Green Fluorescent Protein (GFP) Transgenic Mice *abs#219*

2:36 **Rebecca Robker**
TGFβ1 deficient mice exhibit impaired follicle growth and luteal maintenance *abs#220*

2:48 **Hsiao Yun Yang**
Effects of Exogenous Gonadotrophin Stimulation on Ovarian Tissue Grafts in the Mouse *abs#221*

3:00 **Megan Lloyd**
Characterisation of the infertility effect induced by a recombinant murine cytomegalovirus expressing murine zona pellucida 3 *abs#222*

3:12 **Sean O'Leary**
Ovarian pathology in mice following immunisation with recombinant murine cytomegalovirus expressing murine zona pellucida 3 *abs#223*

Session 11 - SRB orals #4 -Implantation

2:00 PM - 3:30 PM Harbourside Rm 4

Chairs: Sarah Robertson and Eva Dimitriadis

2:00 **Natalie Hannan**
Expression of chemokines and their receptors at the human maternal-embryonic interface *abs#225*

2:15 **Melinda Jasper**
Regulated expression of adhesion and anti-adhesion molecules in mouse endometrium during early pregnancy *abs#226*

2:30 **Evdokia Dimitriadis**
An inhibitor of leukemia inhibitory factor signalling blocks embryo implantation in the mouse *abs#227*

2:45 **Hidetaka Okada**
Progestin-induced proprotein convertase 6 is necessary for decidualization of human endometrial stromal cells *in vitro* *abs#228*

3:00 **David Sharkey**
Seminal plasma TGFβ activates pro-inflammatory cytokine synthesis in human cervical epithelial cells *abs#229*

3:15 **Megan Bray**
Novel immune modulation to improve reproductive outcomes in pigs *abs#230*

Afternoon Tea

3:30 PM - 3:45 PM Exhibition Area

Session 12 - ESA Symposium, Diverse Roles of Estrogen in Males

3:45 PM - 5:45 PM Harbourside Auditorium

Session sponsored by MaynePharma

Chair: Evan Simpson and Rory Clifton-Bligh

3:45 **Renea Jarred-Taylor**
Estrogen action in the prostate *abs#017*

4:15 **Wah Chin Boon**
The role of estrogens in the testis and the male brain – lessons from knockout mice *abs#018*

4:45 **David Celermajer**
Estrogens, Androgens and Cardiovascular Disease in Man *abs#019*

5:15 **Natalie Sims**
Estrogen Receptors in bone: insights from knockout mice *abs#020*

Session 13 - ESA orals #1 - GH/IGF

3:45 PM - 5:45 PM Harbourside Rm 2

Chair: Sue Firth

3:45 **Vincenzo Russo**
Amino acid substitutions in IGFBP-2 alter binding to extracellular matrix components and affects neuronal cells responses to growth and migration *abs#107*

4:00 **Sue Firth**
Role of insulin-like growth factor binding protein-5 ternary complexes in insulin-like growth factor-i bioavailability *abs#108*

4:15 **Sophie Chan**
Insulin-like growth factor binding protein-3 (IGFBP-3) inhibits insulin stimulated glucose uptake in adipocytes *abs#109*

4:30 **Kin Chuen Leung**
Oestrogen and Selective Oestrogen Receptor Modulators (SERM) Exert Divergent Effects on Growth Hormone Signalling Through Different Mechanisms *abs#110*

4:45 **Chris Howe**
IGF-I, IGFBP-3 and ALS as potential markers of growth hormone doping in elite athletes effects of demographic factors and sporting type *abs#111*

5:00 **Gordon Howarth**
IGF-I administration decreases severity of chemotherapy-induced mucositis via modulation of epithelial cell dynamics *abs#112*

5:15 **Kin Chuen Leung**
In Vivo Regulation of Gene Expression by Growth Hormone in Muscle from Hypopituitary Men: A Microarray Study *abs#113*

5:30 **Liping Chung**
Detection of novel biomarkers of human growth hormone administration using SELDI-TOF mass spectrometry *abs#114*

5:45 **Gordon Howarth**
Colonic injury and repair in Dipeptidyl Peptidase IV Knockout Mice with experimental colitis induced by dextran sulphate sodium *abs#115*

Session 14 - ESA orals #2 - Parturition

3:45 PM - 5:45 PM Harbourside Rm 6

Chair: Vicki Clifton

3:45 **Susan Hisheh**
Marked upregulation of COX-2 expression in rat placenta during late pregnancy *abs#116*

4:00 **Renee Johnson**
Proteomic Analysis of the Human Amnion and Chorion at Normal and Preterm Labour *abs#117*

4:15 **Vicki Clifton**
Sex specific mechanisms of human fetal cortisol metabolism and immunity *abs#118*

4:30 **Toni Welsh**
Progesterone receptor mRNA abundance in the gestational tissues of the guinea pig *abs#119*

4:45 **Tamas Zakar**
Mechanisms of Prostaglandin Synthesis Regulation in the Fetal Membranes *abs#120*

5:00 **Richard Nicholson**
Steroid hormone regulation of placental CRH gene expression: A complex mix of molecular mechanisms *abs#121*

5:15 **Amanda Johnston**
Repeated maternal glucocorticoid treatment reduces renal glucocorticoid receptor protein levels in fetal sheep *abs#122*

5:30 **Damien Hewitt**
Placental expression of PPARδ, PPARγ and RXRα in rat pregnancy and the effects of increased glucocorticoid exposure *abs#123*

Session 15 - SRB orals #5 - Embryo

3:45 PM - 5:45 PM Harbourside Rm 4

Chairs: Marie Pantaleon and Michelle Lane

3:45 **Michelle Lane**
Ammonium affects mitochondrial distribution and function in mouse 2-cell embryos *abs#233*

3:57 **Marie Pantaleon**
Calcium involvement in glucose induced GLUT3 expression in preimplantation mouse embryos *abs#234*

4:10 **Yan Li**
PAF induced changes in intracellular Ca^{2+} and membrane potential in the 2-cell mouse embryo *abs#235*

4:22 **Sarah Jansen**
Differential expression of monocarboxylate cotransporter proteins in preimplantation embryos *abs#236*

4:36 **Fan-chin (Emmy) HUNG**
Insulin receptor expression in mouse preimplantation embryos *abs#237*

4:48 **Alexandra Harvey**
Determination of differentially displayed oxygen-sensitive genes in bovine blastocysts *abs#238*

5:00 **Veena Naik**
Sensitivity of bovine morulae and blastocysts to heat shock in vitro *abs#239*

5:12 **Chris O'Neill**
Fertilization *in vitro* causes precocious activation of transcription from the zygotic genome *abs#240*

5:24 **David Kennaway**
Reproductive performance in *Clock* mutant mice *abs#241*

5:36 **Maria Butler**
Stress applied to ewes at Day 2 and 3 of gestation increases Day 6 embryo cell counts *abs#242*

Session 16 - Joint ESA-SRB orals, Male Reproduction

3:45 PM - 5:45 PM Harbourside Rm 3

Chair: David Handelsman and Mark Hedger

3:45 **Alison Death**
Tetrahydrogestrinone (THG) is a potent androgen and progestin *abs#124*

4:00 **Paul Farnworth**
Differential regulation of inhibin binding via betaglycan expression in several mouse cell lines *abs#125*

4:15 **Claire Kennedy**
A repository of ENU mutant mouse lines and their potential for male fertility research *abs#126*

4:30 **Amanda Beardsley**
An $\alpha_6\beta_1$-Integrin/Focal Adhesion Kinase complex may regulate spermiation and spermiation failure *abs#127*

4:45 **Anette Szczepny**
Nuclear Transport of GLI Transcription Factors During Spermatogenesis *abs#243*

5:00 **Shaun Roman**
Interaction between bone morphogenetic protein 4 and retinoid signaling in mouse spermatogenesis *abs#244*

5:15 **Wendy Winnall**
Characterisation of prostaglandin production in the normal and inflamed rat testis *abs#245*

5:30 **Christopher Scott**
The effect of testosterone & season on prodynorphin mRNA expression in the preoptic area hypothalamus of the ram *abs#246*

Session 17 - ESA AGM

5:40 PM - 5:45 PM Harbourside Auditorium

Chair: Ben Canny

Session 18 - SRB Student Meeting

5:45 PM - 6:15 PM Harbourside Rm 4

Chair: Amanda Sferuzzi-Perri

Session 19 - Student BBQ

7:30 PM - 11:30 PM Blackbird Cafe

Tuesday, 24 August 2004

Session 20 - SRB Plenary

8:30 AM - 9:30 AM Harbourside Auditorium

Session sponsored by Meat & Livestock Australia

Chairs: Rob Banks, Meat and Livestock Australia

Thomas Spencer
Uterine and placental factors regulating conceptus growth: insights from the ewe *abs#003*

Session 21 - ESA orals #4 - Male Reproduction (II)

8:30 AM - 9:30 AM Harbourside Rm 4

Chair: Moira O'Bryan

8:30 **Jonathan Beilin**
A population based study of the age related decline in Androgen levels in Australian men *abs#132*

8:45 **Jennifer Spaliviero**
Development and validation of modified Silastic implants to deliver physiological blood estradiol (E2) concentrations in mice *abs#133*

9:00 **Huong Van Nguyen**
Testosterone levels in men with chronic hepatitis C infection: effect of disease severity and recombinant interferon-alpha therapy *abs#134*

Session 22 - ESA orals #5 - Targeted Gene Knockouts; G Proteins

8:30 AM - 9:30 AM Harbourside Rm 5

Chair: Jenny Favaloro

8:30 **Helen MacLean**
Generation of skeletal muscle-specific androgen receptor knockout mice *abs#136*

8:45 **Jeffrey Zajac**
Generation of an androgen receptor knockout mouse line using the Cre/loxP system *abs#137*

9:00 **Chen Chen**
Regulation of the expression of orexin receptors (1 and 2) and leptin receptors (A and B) by oestrogen in the aromatase knock-out (ArKO) mouse *abs#138*

9:15 **Matthew Dalrymple**
Functional characterisation of orexin receptor subtypes 1 and 2 using bioluminescence resonance energy transfer (BRET) and confocal microscopy *abs#139*

9:30 **Kevin Pfleger**
A comparative study of Renilla luciferase substrates and their application to real-time bioluminescence resonance energy transfer (BRET) *abs#140*

Morning Tea

9:30 AM - 10:00 AM Exhibition Area

Break sponsored by GlaxoSmithKline

Session 23 - ESA orals #6 - Mayne Award and Clinical (I)

10:00 AM - 12:15 PM Harbourside Auditorium

Session sponsored by Mayne Pharma

Chair: Ross Cuneo and Ben Canny

10:00 **Abdullah Omari**
The metabolic syndrome in asia: prevalence and risk factors *abs#141*

10:15 **Sonia Davison**
Changes in androgen levels across the adult female life span and relationships to sexual function *abs#142*

10:30 **Caroline Meyer**
Vascular dysfunction and metabolic parameters in PCOS *abs#143*

10:40 **Melissa Gillett**
Management of subclinical hypothyroidism - an audit *abs#144*

10:50 **John Walsh**
Thyroid dysfunction as a predictor of cardiovascular disease and mortality: An analysis from the Busselton Health Study *abs#145*

11:00 **Anthony O'Sullivan**
Amiodarone-induced thyrotoxicosis: factors which effect recovery and outcome *abs#146*

11:10 **Diana Learoyd**
Genotype-phenotype analysis and extensive experience of prophylactic thyroidectomy in a very large multiple endocrine neoplasia type 2A kindred with the RET codon 804 mutation *abs#147*

11:20 **Kym Rae**
Maternal Serum Follistatin Concentrations are Influenced by Foetal Sex *abs#148*

11:30 **Walter Plehwe**
Familial Papillary Thyroid Carcinoma *abs#149*

Session 24 - ESA orals #7 - Signalling

10:00 AM - 12:00 PM Harbourside Rm 5

Chair: Kin Leung

10:00 **Ross Bathgate**
Investigations into the Domains of the Relaxin-1 (LGR7) and INSL3 (LGR8) Receptors Responsible for Ligand Binding and Activation *abs#150*

10:15 **Craig Harrison**
Antagonism of activin type II receptors *abs#151*

10:30 **Tertia Purves-Tyson**
Estrogen actions and signalling mechanisms in cultured rat pelvic autonomic and sensory neurons *abs#152*

10:45 **Gary Leong**
Oestrogen stimulates hepatic expression of suppressors of cytokine signalling-2 and -3 *abs#153*

11:00 **Yao Wang**
Transforming growth factor (TGF)- β can regulate inhibin binding via betaglycan expression in mouse cell lines *abs#154*

11:15 **Ruth Escalona**
Differential actions of bambi on TGFβ superfamily member signalling *abs#155*

11:30 **Dave Grattan**
Prolactin-induced phosphorylation of CREB in gonadotrophin-releasing hormone neurons in mice *abs#156*

Session 25 - Joint ESA-SRB orals, Female Reproduction

10:00 AM - 12:00 PM Harbourside Rm 2

Chair: Darryl Russell

10:00 **Kara Britt**
Effects of phytoestrogens on the ovarian and pituitary phenotypes of estrogen deficient female Aromatase knockout mice *abs#158*

10:15 **Andrea Cussons**
Polycystic ovary syndrome: are endocrinologists and gynecologists treating the same patients? *abs#159*

10:30 **Robert Norman**
Ovarian leukocytes and cytokines in polycystic ovary syndrome *abs#160*

10:45 **Robert Gilchrist**
Molecular basis of oocyte-paracrine signalling that promotes mouse granulosa cell proliferation *abs#247*

11:00 **Rebecca Jones**
Activin A Upregulates Endometrial Metalloproteases: Potential Mechanisms for Promotion of Decidualisation and Implantation *abs#249*

11:15 **Ashwini Chand**
Chemokine Profiling in Endometriosis using Laser Capture Microdissection *abs#250*

11:30 **Sonia Davison**
Measurement of Total Testosterone in Women: Comparison of a direct radioimmunoassay versus radioimmunoassay after organic solvent extraction and Celite partition column chromatography *abs#161*

Session 26 - SRB Symposium: Cell Biology of Male Germ Cells

10:00 AM - 12:00 PM Harbourside Rm 4

Session sponsored by ARC Centre of Excellence in Biotechnology and Development

Chairs: John Aitken and Andrew Sinclair

10:00 **Peter Koopman**
Identification and study of genes important for fetal germ cell biology in mice *abs#021*

10:30 **Stefan Schlatt**
Spermatogonial stem cells: from basic research to clinical applications *abs#022*

11:00 **Kate Loveland**
Drivers of germ cell differentiation *abs#023*

11:30 **Richard Ivell**
Postmeiotic gene transcripts - sense, antisense, or nonsense. *abs#024*

Session 27 - ESA Harrison Lecture

12:00 PM - 1:00 PM Harbourside Auditorium

Chair: Ben Canny

Bruce McEwen
Stress, homeostasis, allostasis and the brain *abs#004*

Lunch

1:00 PM - 2:00 PM Exhibition Area

Break sponsored by Novartis

Session 28 - SRB Symposium: Genetic Strategies in Unravelling Reproductive Disorders

1:30 PM - 3:00 PM Harbourside Auditorium

Session sponsored by Novartis

Chairs Grant Montgomery and Moira O'Bryan

1:30 **Andrew Shelling**
Ovaries: up in a POF of smoke *abs#025*

2:00 **Grant Montgomery**
Endometriosis - Linkage, Positional cloning and Genome wide association *abs#026*

2:30 **Graham Mann**
Mapping novel breast cancer susceptibility genes by linkage analysis of Australian multiple case kindreds *abs#027*

Session 29 - ESA Posters #2

2:00 PM – 3:00 PM Harbourside Foyer

2nd viewing session for posters

Session 30 - ESA Symposium (Neuroendocrinology): "Estrogen and its Effects in the Brain"

3:00 PM - 5:00 PM Harbourside Rm 4

Session sponsored by DSL Labs

Chairs: Brian Oldfield and Iain Clarke

3:00 **Bruce McEwen**
Neurobiology of Ovarian Steroids: "It Ain't Just Sex Anymore" *abs#028*

3:30 **Naomi Rance**
Reproductive Aging and the Human Hypothalamus *abs#029*

4:00 **Allan Herbison**
Mechanisms of estrogen–induced plasticity in GnRH neurons *abs#030*

4:30 **Iain Clarke**
Estrogen feedback regulation to gonadotropin releasing hormone (GnRH) cells involves seria and converging pathways; studies in the sheep *abs#031*

Afternoon Tea (SRB only)

3:00 PM - 3:15 PM Exhibition Area

Session 31 - ESA orals #8 - Clinical (II)

3:00 PM - 5:00 PM Harbourside Rm 2

Session sponsored by Servier

Chair: Bruce Robinson

3:00 **Anna Story**
Effect of sex steroids on BMD, anthropometry and lipids in transsexual subjects *abs#162*

3:15 **Anne Nelson**
Variation of bone and connective tissue turnover markers with age, gender, BMI and ethnicity in elite athletes *abs#163*

3:30 **Suresh Varadarajan**
Frankston Bone Protection Project: Failure of Inpatient and Outpatient Management of Osteoporosis 12 months after a Fragility Fracture *abs#164*

3:45 **Cynthia Ong**
Spinal Cord Compression Secondary to Marked Angulation of the Spine after Percutaneous Vertebroplasty – A Case Report *abs#165*

4:00 **Frances Milat**
Two Cases of Hyperandrogenism in the Setting of Leflunomide (Arava) Therapy *abs#166*

4:15 **Alison Venn**
Oestrogen treatment to reduce the adult height of tall girls: How well did it work? *abs#167*

4:30 **Udo Meinhardt**
Quantifying long-range correlation properties of glycemic control - method and physiological impacts *abs#168*

4:450 **Leo Turner**
Determinants of Testicular Function and Bone Density in Parkinson's Disease *abs#169*

Session 32 - ESA orals #9 - Adipocytokines & Metabolism

3:00 PM - 5:00 PM Harbourside Rm 5

3:00 **Leon Brownrigg**
Differential modulation of cell cycle progression and apoptosis by synthetic and endogenous PPAR gamma ligands in monocytic cells *abs#170*

3:15 **DanDan Feng**
Signal transduction pathway involved in free fatty acid-induced reduction of voltage-gated K^+ currents in primary cultured rat pancreatic β-cells *abs#171*

3:30 **Kheng Tan**
Regulation of gluconeogenic enzymes by glucocorticoids in glucocorticoid receptor-null mice *abs#172*

3:45 **Jenny Gunton**
Altered gene expression in pancreatic islets of humans with type 2 diabetes mellitus *abs#173*

4:00 **David Kennaway**
Disruption of the clock gene *BMAL*1 alters glycaemia and circulating adipocytokines in mice and induces hypoinsulinaemia in males *abs#174*

4:15 **Rebecca Robker**
Adipose Tissue Production of sICAM-1 in a Murine Model of Obesity: Gender Differences in Response to a High Fat Diet *abs#175*

4:30 **Stephen Twigg**
Regulation of Connective Tissue Growth Factor in Adipose Tissue by the Metabolic Syndrome and Thiazolidenedione *abs#176*

4:45 **Peter Mark**
Plasma leptin-binding activity increases from pre-puberty to adulthood in male rats *abs#177*

Session 33 - SRB New Investigator Award and MLA Award Finalists

3:15PM - 5:00 PM Harbourside Auditorium

Session sponsored by Serono & Meat and Livestock Australia

Chair: Lois Salamonsen

3:15 **Rebecca Dragovic**
Regulation of mouse cumulus expansion by oocyte-secreted growth differentiation factor-9 (GDF-9) *abs#251*

3:30 **Tamer Hussein**
Oocyte-secreted factor(s) regulate apoptosis of bovine cumulus cells *abs#252*

3:45 **Simon Degen**
Follicle stimulating-hormone (FSH) withdrawal induces germ cell apoptosis in the immature rat testis *in vitro* *abs#253*

4:00 **Christine White**
Interleukin-11 enhances endometrial stromal cell decidualization via activation and inhibition of target genes *abs#254*

4:15 **Kylie Webster**
Dnmt3L; a coordinator of epigenetic modifications during spermatogenesis *abs#255*

4:30 **Rachel Chan**
Development of a Label Retaining Cell Method to Identify Stem Cells in Mouse Endometrium *abs#256*

4:45 **Leanne Cotton**
Fibroblast growth factor receptor-1 (FGFR-1) is essential for spermiogenesis and male fertility *abs#257*

Session 34 - ESA Taft Lecture

5:00 PM - 6:00 PM Harbourside Auditorium

Chair: Ross Cuneo

Peter Clayton
Translating Growth Hormone Secretion into Growth *abs#005*

Session 35 - SRB AGM

5:00 PM - 6:00 PM Harbourside Rm 4

Chair: Lois Salamonsen

ESA/SRB Conference Dinner

7:00 PM - 11:00 PM Harbourside Function Room

Wednesday, 25 August 2004

Session 36 - SRB orals #6 - Male Reproductive Tract

8:30 AM - 9:30 AM — Harbourside Rm 3

Chairs: Wendy Winnall and Shaun Roman

8:30 **David Aridi**
Natural Killer Cell Activity in the Adult Rat Testis *abs#258*

8:40 **Megan Crane**
Identification of elevated levels of apoptosis among T-cells isolated from the rat testis *abs#259*

8:50 **Katerina Dvorakova**
Sperm trains and mating behaviour in wood mice of the genus apodemus *abs#260*

9:00 **Sarah Robertson**
Probiotic lactobacillus in seminal plasma *abs#261*

9:10 **Russell Jones**
How the elephant got its epididymis *abs#262*

9:20 **Christopher Grupen**
Effects of animal pairing on marmoset sperm collected by penile vibratory stimulation *abs#263*

Session 37 - SRB orals #7 – Placenta and Partuition

8:30 AM - 9:30 AM — Harbourside Rm 4

Chairs: Julie Owens and Sally Dunwoodie

8:30 **Padma Murthi**
Role of homeobox gene Hlx expression in normal placental developmemt *abs#271*

8:42 **Amanda Sferruzzi-Perri**
Insulin-like growth factor treatment of pregnant guinea pigs during early pregnancy promotes fetal growth *abs#273*

8:54 **Julie Owens**
Increased perinatal mortality following restriction of placental and fetal growth *abs#285*

9:06 **Cameron Nowell**
Differential Expression of Plasminogen Activation Cascade Components in Human Preterm Delivery with and without Preterm Premature Rupture of the Fetal Membranes *abs#287*

9:18 **Alfia Alghafra**
Labour-associated changes in human fetal membranes and placental adrenomedullin mRNA expression *abs#288*

Session 38 - ESA, ADS & ADEA Joint Plenary

8:30 AM - 9:30 AM — Harbourside Auditorium

Session sponsored by Servier

Chair: Glen Ward & Ben Canny

Phillip Scherer
The physiology of adipose tissue in the diabetic state: influence on carbohydrate and lipid metabolism, inflammation and cancer *abs#006*

Session 39 - ESA orals #3 - Female Reproduction (II)

8:30 AM - 9:30 AM Harbourside Rm 2

Chair: Rebecca Robker

8:30 **Evdokia Dimitriadis**
Relaxin, progesterone and prostaglandin E_2 regulate interleukin 11 during human endometrial stromal cell decidualization *abs#128*

8:45 **Theresa Hickey**
Expression of prostate specific antigen by ovarian granulosa cells *abs#129*

9:00 **Christopher Haines**
Early luteal phase progesterone supplementation and IVF-ET outcome *abs#130*

9:15 **Karla Estrada**
Gene expression of Orexin, Melanin-concentrating hormone and Neurotensin across the estrous cycle of ewe *abs#131*

Morning Tea

9:30 AM - 10:00 AM Exhibition Area

Session 40 - ESA & ADS Symposium (clinical): Hormones and Growth

10:00 AM - 12:00 PM Pyrmont 1&2

Session sponsored by Novo Nordisk

Chair: Patricia Crock and Shaun McGrath

10:00 **Peter Clayton**
Growth Hormone Deficiency – the Clinical Spectrum and Treatment Options *abs#032*

10:30 **Bruce King**
The role of IGF-1 in clinical practice *abs#033*

11:00 **Margaret Zacharin**
Optimizing growth and hormone replacement in Turner syndrome *abs#034*

11:300 **Andrew Cotterill**
Thyroid disorders in children and adolescence *abs#035*

Session 41 - ESA & ADS Symposium (basic), Adipocytokines and Signalling

10:00 AM - 12:00 PM Harbourside Rm 3

Session sponsored by GlaxoSmithKline

Chair: Greg Cooney and Paul Williams

10:00 **Phillip Scherer**
Adiponectin and Resistin: Structure, Function and Regulation of Bioactivity *abs#036*

10:30 **David Stapleton**
AMP-activated protein kinase: Linking adipocytokine signaling to metabolic control *abs#037*

11:00 **Mark Febbraio**
Interleukin-6 and insulin sensitivity: friend or foe? *abs#038*

11:30 **John Prins**
TNFα *abs#039*

Session 42 - ESA & SRB Joint Symposium: TGFß Superfamily in Reproduction, Development and Pathological States

10:00 AM - 12:00 PM Harbourside Rm 4

Chair: Rob Gilchrist and David Phillips

10:00 **Aaron Hsueh**
Ovarian research in the postgenomic era: analyses of GDF-9 and other paracrine signaling pathways *abs#040*

10:30 **David Phillips**
Activin A: from reproductive factor to inflammatory cytokine *abs#041*

11:00 **John McAvoy**
The role of TGF-beta in normal and pathological lens development *abs#042*

11:30 **Catherine Coulter**
Role of TGFBeta in adrenal steroidogenesis before birth *abs#043*

Lunch

12:00 PM - 1:00 PM Exhibition Area

Session 43 - ESA Lecture

1:00 PM - 2:00 PM Pyrmont 1&2

Session sponsored by GlaxoSmithKline

Chair: Cathie Coulter

Aaron Hsueh
Hormone research based on evolutionary analysis of genomic data *abs#007*

Session 44 - SRB oral #8 - Pregnancy

1:00 PM - 2:00 PM Harbourside Rm 4

Chairs: Jeremy Thompson and Sharon McCracken

1:00 **Budi Marjono**
Macrophage inhibitory cytokine-1 at the maternal-fetal interface in early human pregnancy *abs#270*

1:15 **Sharon McCracken**
Nuclear Factor Kappa B Down-Regulation in human T-cells is Essential for the Maintenance of the Cytokine Profile Required for Pregnancy Success *abs#286*

1:30 **Jonathan McGuane**
Decreased Expression of Estrogen Receptor Beta in the Reproductive Tract of Pregnant Relaxin-Deficient (*Rlx*$^{-/-}$) Mice *abs#272*

1:45 **John Bromfield**
Semen exposure in early pregnancy impacts fetal and neonatal growth *abs#274*

Session 45 - SRB oral #9 - Gene Regulation / Signalling

1:00 PM - 3:00 PM Harbourside Rm 3

Chairs: Kate Loveland and Laura Parry

1:00 **Gurpreet Kaur**
Calmodulin-dependent nuclear import pathway of the testis-determining factor SRY *abs#275*

1:12 **Athina Efthymiadis**
Targets of the action of nuclear transport factors in spermatogenesis *abs#276*

1:24 **Kelly Cunningham-Smith**
The Role of pp60c-src Tyrosine Kinase in Sperm Capacitation *abs#277*

1:36 **Sirisha Mendis**
Bioactive Activin and its Affects on Murine Testis Development *abs#278*

1:48 **Darryl Russell**
Infertility in mice with null mutation of the Egr-1 transcription factor *abs#279*

2:00 **Michael Boden**
Reproductive consequences of circadian dysfunction: Fertility in the *Bmal1* null mouse *abs#280*

2:12 **Mai Sarraj**
A novel scavenger receptor domain containing gene differentially expressed in the embryonic mouse testis *abs#281*

2:24 **Laura Parry**
Differential Expression of the Relaxin Receptor (LGR7) in the Mammary Apparatus of the Lactating Tammar Wallaby (*Macropus eugenii*) *abs#282*

2:36 **Jon Curlewis**
Effects of prostaglandins on SOCS expression in T-47D breast cancer cells *abs#283*

2:48 **Marissa Bowden**
Expression of HTRA1, 2 and 3 in human endometrial cancer *abs#284*

Session 46 - SRB orals #10 – Endometrial Biology

2:00 PM - 3:00 PM Pyrmont 1&2

Chairs: Caroline Gargett and Melinda Jasper

2:00 **Lisa Walter**
The effects of progesterone on endometrial angiogenesis in pregnant and ovariectomised mice *abs#264*

2:10 **Tu'uhevaha Kaitu'u**
Matrix metalloproteinases in the mouse model of menstruation: effect of doxycycline inhibition *abs#265*

2:20 **Anna Ponnampalam**
Human endometrial cycle stages can be determined by global gene expression profiling *abs#266*

2:30 **Naomi Morison**
Leukocyte matrix metalloproteinase expression is regulated in response to selected chemokines: implications for break-through bleeding *abs#267*

2:40 **Kjiana Schwab**
Identifying markers for stromal stem/progenitor cells in human endometrium *abs#268*

2:50 **Marina Zaitseva**
Estrogen and Progesterone Receptor Expression is significantly Reduced in cultured Myometrial and Fibroid Smooth Muscle Cells *abs#269*

Session 47 - ESA orals #10 - Cancer

2:00 PM - 4:00 PM Harbourside Rm 2

Chair: Deborah Marsh and Rebecca Jones

2:00 **Premila Paiva**
Expression patterns of hyaluronan, hyaluronan synthases and hyaluronidases suggest a role for hyaluronan in the progression of endometrial cancer *abs#178*

2:15 **Kelly Avery**
Co-localisation of the progesterone receptor with brca1 in human breast cancer cells *abs#179*

2:30 **Simon Chu**
NF-κB signalling in granulosa cell tumours *abs#180*

2:45 **Christin Down**
Role of the Hu proteins, HuR AND HuD, in the regulation of androgen receptor expression and activity in prostate cancer cells *abs#181*

3:00 **Neveen Tawadros**
Roles for Ghrelin in Promoting Endometrial Adenocarcinoma *abs#182*

3:15 **Nirusha Arnold**
Dilemmas in the Management of Patients with Differentiated Thyroid Carcinoma *abs#183*

3:30 **David Henley**
Heterogeneity of familial paraganglioma syndromes in four patients with SDHB mutations *abs#184*

Session 48 - ESA orals #11 - In Utero and Postnatal Endocrine Influences

2:00 PM - 4:00 PM Harbourside Rm 4

Chair: Roger Smith and Cathy Gatford

2:00 **Kathryn Gatford**
Restriction of placental and fetal growth reduces growth hormone pulse frequency in neonates *abs#186*

2:15 **Simon Forehan**
The role of AIRE in thymocyte development in a murine model of Type 1A diabetes *abs#187*

2:30 **Margaret Zacharin**
Vitamin D deficiency in pregnancy: postnatal audit of vitamin D and bone health in women and their infants *abs#188*

2:45 **Fran Leone**
Placental restriction impairs glucose homeostasis in adult rats, independently of adiposity and circulating free fatty acids *abs#189*

3:00 **Tien Hng**
Thyroid hormone metabolism and birth weight *abs#190*

3:15 **Deborah Sloboda**
Prenatal glucocorticoid exposure alters long-term hepatic protein expression in sheep *abs#191*

3:30 **Julie Owens**
Placental restriction and small size at birth accelerate the increase in plasma leptin with age in the sheep *abs#192*

3:45 **Julie Owens**
Sex differences in impaired glucose homeostasis in the young adult guinea pig with spontaneous fetal growth restriction *abs#193*

AUTHOR INDEX by abstract number

INVITED SPEAKERS

001

INTRACRINOLOGY OF HUMAN BREAST CANCER – INTRATUMORAL ESTROGEN PRODUCTION

H. Sasano

School Of Medicine, Tohoku University, Sendai, Japan

Recently various experimental and clinical findings have demonstrated that in situ production of sex steroids through conversion of circulating biologically inactive precursors to active hormones, has been considered to play very important roles in pathogenesis and/or development of sex steroid dependent carcinoma such as breast, endometrial, ovarian and prostate cancer. This in situ production of sex steroids such as dihydrotestosterone or estradiol in carcinoma tissues can provide amounts of steroids sufficient to bind to their corresponding receptors and yield subsequent steroid dependent hormonal actions regardless of serum concentrations of biologically active steroids. This is especially evident in the cases of development and/or progression of estrogen dependent or estrogen receptor positive breast carcinoma in post menopausal women. This mode of actions of sex steroids is termed " Intracrinology" in contrast to classical "Endocrinology". This concept of "Intracrinology" plays very important roles not only in understanding pathogenesis and/or biological features of estrogen dependent breast cancer but also in its endocrine treatment. In this presentation, the mechanisms of intracrine or intratumoral estrogen production in human breast cancer will be first presented with emphasis on interactions of various enzymes involved in this biosynthesis such as aromatase, 17beta-hydroxysteroid dehydrogenase, estrogen sulfatase, estrogen sulfotransferase and others. The possible regulation of intratumoral estrogen production in human breast cancer will be then presented mainly through the regulation of the expression of these estrogen producing and/or metabolizing enzymes above including the possible involvement of various nuclear receptors. Finally biological and/or clinical relevance of intratumoral or intracrine estrogen production including the application of aromatase inhibitors in postmenopausal women with breast cancer will be discussed.

002

HUMAN SPERMATOZOA: FRUITS OF CREATION, SEEDS OF DOUBT

R. J. Aitken

Biological Sciences, University of Newcastle, Callaghan, NSW, Australia

Defective sperm function is the largest defined cause of human infertility, affecting one in twenty Australian males. Despite its prevalence, we are only just beginning to understand the underlying mechanisms. The past decade has seen two major advances in this field: (1) the discovery that Y chromosome deletions play a key role in the aetiology of non-obstructive azoospermia/oligozoospermia (2) recognition that oxidative stress can impact upon the functional competence of human spermatozoa through peroxidative damage to the sperm plasma membrane. Oxidative stress has also been found to disrupt the integrity of DNA in the male germ line and may represent an important mechanism by which environmental impacts on human health are mediated. Thus, paternal exposure to various toxicants (cigarette smoke, organic solvents, heavy metals) has

been linked with oxidative DNA damage in spermatozoa and developmental defects, including cancer, in the F1 generation. The male germ line becomes particularly vulnerable to such factors during the post meiotic stages of differentiation. Pre-meiotic germ cells always have the option of undergoing apoptosis if DNA damage is severe. However, post-meiotic germ cells have lost both the ability to mount an apoptotic response and the capacity for DNA repair. As a result, germ cells are particularly vulnerable to genotoxic agents during spermiogenesis and epididymal maturation. If the fertilizing capacity of the spermatozoa is retained following toxicant exposure, then DNA damage will be transferred to the zygote and must be repaired subsequently by the oocyte and/or early embryo. Aberrant DNA repair at this stage has the potential to create mutations that will compromise embryonic development and, ultimately, the normality of the offspring. Elucidating the causes of oxidative damage in spermatozoa should help resolve the aetiology of conditions such as male infertility, early pregnancy loss and childhood disease, including cancer.

003

UTERINE AND PLACENTAL FACTORS REGULATING CONCEPTUS GROWTH: INSIGHTS FROM THE EWE

T. E. Spencer

Animal Science, Texas A&M University, 2471 Tamu, Texas, United States

Uterine adenogenesis is the process whereby endometrial glands differentiate and develop and is primarily a postnatal event in all mammals. In domestic animals and humans, adenogenesis involves initial differentiation and budding of glandular epithelium followed by invagination and extensive tubular coiling and branching morphogenesis through the endometrial stroma to the myometrium. Transient exposure of the neonatal ewe to a progestin from birth to postnatal day 56 resulted in a uterine gland knock out (UGKO) phenotype in the adult. UGKO ewes exhibit a peri-implantation defect in conceptus (embryo/fetus and associated extraembryonic membranes) survival, indicating the functional importance of uterine glands and their secretions. Genomic and proteomic analysis of uterine endometrium from UGKO ewes has identified many candidate genes that regulate conceptus development and implantation, including endogenous Jaagsiekte sheep retroviruses (enJSRVs), glycosylated cell adhesion molecule one (GlyCAM-1), osteopontin, and galectin-15. Galectin-15, also known as OVGAL11 and a previously uncharacterized member of the galectin family of secreted beta-galactoside lectins, was discovered in the endometrium of sheep. In endometria of cyclic and pregnant sheep, galectin-15 mRNA was expressed specifically in the endometrial luminal epithelium but not in the conceptus. In pregnant sheep, galectin-15 mRNA expression appeared in the epithelia between days 10 and 12 and increased between days 12 and 16. Progesterone induced and intereron tau stimulated galectin-15 mRNA in the endometrial epithelium. Galectin-15 protein was concentrated near and on the apical surface of the endometrial luminal epithelia and localized within discrete cytoplasmic crystalline structures of conceptus trophectoderm. Galectin-15 is hypothesized to function extracellularly to regulate trophectoderm migration and adhesion to the endometrial epithelium and intracellularly to regulate cell survival, growth, and differentiation. In sheep, the sequential actions of ovarian steroid hormones (estrogen and progesterone), interferon tau, placental lactogen and placental growth hormone constitute a servomechanism that directly regulates endometrial gland morphogenesis and terminal differentiated function to provide increasing histotrophic nutrition for conceptus growth and development. Knowledge gained from this research will be used to prevent or treat infertility, fetal growth retardation, and disease in domestic animals and humans.

004

FROM MOLECULES TO MIND: STRESS, ALLOSTASIS AND INTEGRATION OF BRAIN AND BODY

B. McEwen

Laboratory of Neuroendocrinology, The Rockefeller University, New York, United States

The mind involves the whole body and two-way communication between the brain and the cardiovascular, immune and other systems via neural and endocrine mechanisms. Stress is a condition of the mind and a factor in the expression of disease that differs among individuals. A broader view is that it is not just the dramatic stressful events that exact their toll but rather the many events of daily life that elevates activities of physiological systems so as to cause some measure of wear and tear. We call this wear and tear "allostatic load", and it reflects not only the impact of life experiences but also genetic load; individual life-style habits reflecting items such as diet, exercise and substance abuse; and developmental experiences that set life-long patterns of behavior and physiological reactivity.(1) Hormones associated with stress and allostatic load protect the body in the short-run and promote adaptation, but the long run allostatic load causes changes in the body that lead to disease. This will be illustrated for the immune system and brain regions involved in stress, fear and cognition (eg, hippocampus, amygdala and prefrontal cortex). Besides developmental influences associated with mother-infant interactions, the most potent of stressors in adult life are those arising from competitive interactions between animals of the same species, leading to the formation of dominance hierarchies. Psychosocial stress of this type not only impairs cognitive function of lower ranking animals, but it can also promote disease (e.g., atherosclerosis) among those vying for the dominant position, as well as depressive illness. Social ordering in human society is also associated with gradients of disease, with an increasing frequency or mortality and morbidity as one descends the scale of socioeconomic status (SES) that reflects both income and education. Although the causes of these gradients of health are very complex, they are likely to reflect, with increasing frequency at the lower end of the scale, the cumulative burden of coping with limited resources and negative life events as well as differences in life style, and the allostatic load that this burden places on the physiological systems involved in adaptation and coping.

(1) McEwen, B.S. Protective and damaging effects of stress mediators. New England J. Med. 238: 171-179 (1998).

HORMONE RESEARCH BASED ON EVOLUTIONARY ANALYSIS OF GENOMIC DATA

A. J. Hsueh

Stanford University, Stanford, California, United States

Recent publication of the entire genomic sequences for human and multiple model organisms has allowed the elucidation of the evolutionary origins of human genes. For hormone researchers, this breakthrough facilitates the analysis of polypeptide ligand and receptor genes by tracing their phylogenetic roots.

Using GenBank searches and an evolutionary approach, we identified new polypeptide hormones and receptors. Because of the coevolution of polypeptide ligands and their cognate receptors, analysis of human genomic sequences allows one to predict the pairing of these elements. Initially, we identified novel human paralogs for glycoprotein hormone subunits (alpha 2 and beta 5) and relaxin/insulin-like factors. In addition, we isolated a group of five orphan LGRs (leucine-rich repeat-containing G protein-coupled receptors) homologous to LH, FSH, and TSH receptors. Matching the polypeptide hormones and receptors led to the finding of a new glycoprotein hormone as an agonistic ligand for the TSH receptor (J Clin Invest., 2002). In addition, receptors for the classic hormone, relaxin, were identified as LGR7 and LGR8 (Science 2002) whereas INSL3 and relaxin 3 were found to be specific ligands for LGR8 and LGR7, respectively (J Biol Chem 2002, 2003). Further, INSL3 was found to mediate the action of the LH in gonads. INSL3 produced by testicular Leydig and ovarian theca cells activates the LGR8 expressed in germ cells to lower intracellular cAMP levels. Treatment with INSL3 induces oocyte maturation and promotes male germ cell survival (PNAS 2004). Based on the evolutionary analysis of genomic data, we also identified the known BMPRII and ALK5 as the receptors for GDF-9 (Biol Reprod 2002; Mol Endo. 2004) and elucidated the downstream signaling pathways in ovarian follicles.

Genomic analysis of the evolution of polypeptide ligand and receptor gene families provides a new paradigm for future identification and matching of novel ligands and receptors. We have set up the Human Plasma Membrane Receptome database at http://receptome.stanford.edu in which more than 1,000 known human receptor gene pages can be accessed and searched online. This database provides a platform for the future analysis of genomic data to elucidate the physiological and pathophysiological roles of diverse polypeptide ligands and receptors.

010

COORDINATING THE TRANSITION FROM EGG TO EMBRYO IN MAMMALS.

J. Carroll

Physiology, UCL, London, United Kingdom

At fertilization of mammalian oocytes, the sperm induces a series of increases in the concentration of intracellular Ca^{2+}. These Ca^{2+} oscillations trigger all the events of egg activation including, cortical granule exocytosis, completion of meiosis and entry into the first mitotic division. Thus, intracellular Ca^{2+} plays a pivotal role in coordinating the transition from egg to embryo. Our work is focussed on understanding how the oocyte prepares for fertilization, how the Ca^{2+} oscillations are controlled and how Ca^{2+} stimulates signalling pathways that lead to optimal early embryonic development. In this lecture I will focus on the downstream pathways of Ca^{2+} signalling at fertilization. Conventional Protein Kinase C (cPKC) is major downstream target of Ca^{2+} in many cell functions. Using PKC-GFP fusion proteins we have found that cPKC is recruited to the membrane in a manner that is dependent on the frequency and amplitude of the Ca^{2+} oscillations. Recruitment of cPKC appears to promote Ca^{2+} influx necessary to sustain the generation of long lasting Ca^{2+} oscillations. In other cell types cytosolic Ca^{2+} increases are known to stimulate mitochondrial respiration. We have found that maintenance of resting Ca^{2+} levels and sperm-induced Ca^{2+} oscillations are critically dependent on mitochondrial ATP production: a feature not shared by many cell types. Since Ca^{2+} release increases ATP consumption we investigated whether the Ca^{2+} transients increase mitochondrial activity so as to meet this increase in demand. Monitoring autofluorescence from NADH and flavoproteins reveals that Ca^{2+} transients stimulate a change in redox state of mitochondria, presumably by activating Ca^{2+} sensitive dehydrogenases of the TCA cycle. Thus, through activation of downstream pathways including, PKC, cyclin B degradation and mitochondrial activity, intracellular Ca^{2+} provides a signal that orchestrates the activation of early mammalian development.

011

WAKING UP THE EGG. HOW THE SPERM REGULATES EXIT OUT OF THE MEIOTIC CELL CYCLE

K. T. Jones

Cell and Molecular Biosciences, Newcastle University, Newcastle, Tyne and Wear, United Kingdom

A series of calcium spikes are induced in the mammalian egg cytoplasm at fertilization. These calcium spikes, which last for several hours, are the necessary and sufficient signal that stimulates the egg to escape from arrest at metaphase of the second meiotic division. Metaphase arrest is achieved by preventing the destruction of cyclin B1, the regulatory component of Maturation (M-Phase) Promoting Factor, and securin, which prevents segregation of sister chromatids. Both these proteins are destroyed by tagging with ubiquitin, using an E3 ligase the Anaphase-Promoting Complex (APC). Ubiquitination tags them for proteolysis by the 26S proteasome. Work from my lab has demonstrated that the sperm calcium signal works through activating the APC, not the 26S proteasome. Although we do not know which APC component is affected by calcium, this activation appears specific to a metaphase-arrested cell cycle state. More recently we have found that the APC is differently regulated at specific points during exit from meiosis II. Before extrusion of the second polar body it is the APC

activator cdc20 that regulates APC activity. However following extrusion of the second polar body cdh1 appears the major regulator. It is probable, therefore, that the calcium spiking affects the activity of both APC^{cdc20} and APC^{cdh1}. This swap in APC activator at the time of second polar body extrusion has not been reported in eggs of other species, in fact non-mammalian eggs all lack cdh1. Since APC^{cdc20} and APC^{cdh1} have different substrate specificities, the function of APC^{cdh1} in mammalian eggs warrants further investigation.

012

METABOLIC DETERMINANTS OF IMPLANTATION SUCCESS AND PROGRAMMING LONG TERM VIABILITY IN EMBRYOS

J. G. Thompson , K. L. Kind

Research Centre for Reproductive Health, The University of Adelaide, Woodville, SA, Australia

It has long been recognized that energy substrate supply and metabolism are key determinants of early embryo development during in vitro culture. Recently it has been revealed that exposure to suboptimal metabolic environments during early embryo development can "programme" subsequent development, leading to perturbed fetal development. For example, amino acid uptake profiles during early cleavage have been found to predict subsequent embryo development and potentially implantation success. However, the by-product of amino acid metabolism, ammonium, has also been found to significantly alter development, possibly through perturbed methylation of imprinted genes. Our own work has focussed on the role of oxygen availability and subsequent embryo development. Somatic cells respond to changing oxygen concentration by altering intracellular REDOX state (the balance between oxidative and reductive power within a cell), which inturn can alter transcription via REDOX-sensitive transcription factor activity. Furthermore, oxygen is known have direct effects on transcriptional activity via the hypoxia-inducible factors (HIFs), transcription factors whose stability and DNA-binding activity are directly regulated by pO_2, in particular under hypoxic conditions. Using a mouse model, we have demonstrated that reducing pO_2 from 50 mmHg to 15 mmHg during the compaction and blastulation periods alone significantly alters expression patterns of oxygen-sensitive genes (such as glucose transporters), without significantly altering developmental progression to the blastocyst stage. Following transfer, embryos cultured under 15 mmHg O_2, despite similar implantation rates, produced fewer viable and lighter fetuses than in vivo derived control embryos or those cultured in either atmospheric or 50 mmHg pO_2. This demonstrates that mouse embryos are sensitive to changes in their metabolic state during the post-compaction period and that operating through causal pathways, the environment during this period of development can significantly affect subsequent developmental potential. Ironically, bovine embryo development appears to benefit under a low O_2 concentration. Furthermore, HIF protein stability appears to differ between the two species, which may be the underlying cause for the differences in gene expression and developmental competence.

THE REGULATION OF SURVIVAL OF THE PRE-IMPLANTATION EMBRYO

C. O'Neill

Human Reproduction Unit, Royal North Shore Hospital, St. Leonards, NSW, Australia

There are many aspects of the regulation of the growth and survival of the pre-implantation embryo that remain enigmatic. The increasing production of such embryos by assisted reproductive technologies (ART) in human medicine, animal production and conservation biology has highlighted the relatively poor viability of such embryos. Many embryos fail to survive past the normal time of implantation. Population biology theory predicts that any circumstance that results in high death rates within a population creates a potential for genetic selection. This occurs if the surviving individuals have a genetic make up that preferentially favours survival. Since ART clearly favours the survival of some embryos over others, it is a high priority to develop a sound understanding of those factors that normally govern embryo survival and how they may be affected by ART. It raises the question, do embryos that survive ART have a genetic make-up that favours their survival compared to the proportion of the population that does not survive? It is now demonstrated that autocrine and paracrine factors are essential for embryo survival and that these act via the 1-o-phosphatidylinositol-3-kinase (PI3K) survival signalling pathway (1). PI3K activates many downstream pro-survival and anti-apoptotic mediators. ART changes the expression of some of these mediators. Pharmacological and genetic moderation of their expression can influence embryo survival, highlighting potential targets for genetic selection through ART. Studies in appropriate models will allow rational approaches to safety assessment of ART and spawn new strategies for media and procedural design.

(1) Lu, D. P. Chandrakanthan, V. Cahana, A. Ishii, S. O'Neill, C. (2004) Journal of Cell Science 117(8): 1567-1576.

014

NOVEL UTERINE GENES IN REGULATION OF EMBRYO IMPLANTATION

G. Nie , Y. Li , K. Luu , J. Findlay , L. Salamonsen

Uterine Biology Laboratory, Prince Henry's Institute of Medical Res., Melbourne, VIC, Australia

Implantation of the embryo into the maternal endometrium is the first and critical step leading to the establishment of a pregnancy. It has been well established that only during the "window" of implantation, a limited time span when the uterine environment is receptive, can a blastocyst successfully implant into the uterus. The development of uterine receptivity is accompanied by remarkable morphological and physiological changes in the endometrium, and this is primarily driven by the coordinated effects of the ovarian steroid hormones. Uterine tissue remodelling during implantation also contributes significantly to the development of the placenta. Insufficient uterine remodelling causes implantation failure and infertility. To date, the exact molecular events occurring in the uterus during the establishment of receptivity and at the actual site of implantation are still not well understood. We used the mouse as a model and identified a number of previously unrecognised molecules that are uniquely regulated in the early stages of implantation: one of these is proprotein convertase 6 (PC6). The potential importance of these genes and their products in modulating fertility in the primate including the human, was demonstrated by their unique spatial and temporal expression in the endometrium of human and rhesus monkey during the phase of uterine receptivity and at implantation. The importance of the genes for implantation was ultimately confirmed by functional studies *in vivo* using morpholino antisense oligonucleotides. These molecules will be discussed in terms of their identity, expression and functions.

015

DISRUPTED DECIDUALIZATION IN SOCS3 GENE MUTANT MICE

L. Robb , S. Rakar , W. Alexander

The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

Cytokines comprise a large family of secreted glycoproteins that regulate many fundamental biological processes. Cytokine signals are relayed to target cells via binding to cell surface receptors. The receptors signal via members of the Janus kinases (JAKs) and signal transduction and activators of transcription family (STATs). The SOCS proteins negatively regulate cytokine signalling by inhibiting components of the JAK/STAT pathway. Genetically modified mice in which individual SOCS genes are ablated have revealed key biological roles for these proteins. SOCS3 null mice die at midgestation due to placental insufficiency. By embryonic day (E)9.5 there is a marked decrease in the spongiotrophoblast layer and an increase in trophoblast giant cells in SOCS3 null placentae. With increasing gestational age, there is progressive disorganization of the SOCS3 null placental labyrinth. Takahashi et al (EMBO J 22: 372 2003) used tetraploid aggregation to demonstrate that the placental defect was attributable to intrinsic defects in the SOCS3 deficient trophoblast cells or yolk sac endoderm. Based on evidence from in vitro assays, SOCS3 has a role in downstream negative regulation of signalling via a large number of cytokines. To identify the cytokine responsible for the placental phenotype, we crossed SOCS3 null embryos with mice lacking leukemia inhibitory factor (LIF). This rescued the placental phenotype of the SOCS3 null mice, thereby demonstrating that alterations in LIF signalling are responsible for profound abnormalities of the murine placenta.

016

ROLE OF CITED GENES IN PLACENTAL MORPHOGENESIS: STUDIES IN NULL MUTANT MICE

S. L. Dunwoodie , S. L. Withington , D. B. Sparrow , A. N. Scott , J. I. Preis , J. Michalicek

Developmental Biology Program, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia

Cited1 and Cited2 interact with CBP and p300. CBP/p300 bind numerous proteins and evidence exists, for Cited2 at least, that Cited binding prevents the binding of other proteins to CBP/p300. Since CBP/p300 interact with many proteins, can acetylate protein and DNA, and act as a ubiquitin ligase, it is likely that Cited1 and Cited2 function at a number of sites during development. We have generated mice that carry a null mutant allele for each of these genes. Analysis of null mutant embryos demonstrates that both Cited1 and Cited2 are required for normal embryonic development and survival. Although both Cited1 and Cited2 are expressed in the developing embryo and placenta, it appears that abnormal placental development and function is the cause of embryonic death.

The defect that develops in the placentas of Cited1 null mutants is not apparent until late in gestation (16.5dpc). Cited1 null mutants are smaller than controls at birth and die during the early postnatal period. The placentas of these mutants are disorganised, with spongiotrophoblasts projecting in to the labyrinthine layer. In addition, resin casts of the maternal blood spaces within these placentas revealed extremely enlarged blood sinuses. We are searching for factors that could result in the increased size of the maternal blood sinuses.

Cited2 null placentas and embryos are significantly smaller than controls; mutants die 3/4 the way through gestation (15.5dpc). The null mutant placentas have proportionally fewer spongiotrophblasts, trophoblast giant cells, and invasive trophoblasts. In addition, resin casts of fetal vasculature of the placental reveal that the capillary network is underdeveloped. Through the isolation of trophoblast stem (TS) cells we are exploring the possibility that TS cell proliferation and/or differentiation is impaired due to a lack of Cited2. We suspect that the development of the phenotype may relate to the Hypoxia Inducible Factor-1a (HIF1a) transcription factor as Cited2 expression is induced by HIF1 and it acts to negatively regulate its activity.

017

ESTROGEN ACTION IN THE PROSTATE

R. A. Jarred-Taylor , J. J. Bianco , S. J. Ellem , S. J. McPherson , G. P. Risbridger

Monash Institute of Reproduction & Development, Monash University, Clayton, Vic, Australia

The prostate gland is a male accessory sex organ that is a common site of disease in aging men, including benign enlargement and malignancy. Prostate growth, during development and disease, is primarily directed and influenced by androgens as well as locally produced growth factors. Estrogens are prototypical female hormones that are also synthesized in males, and there is significant evidence that the prostate gland is an estrogen-target organ. We have spent several years documenting the biological actions of estrogens on prostate growth.

As in many other tissues, estrogens can act indirectly on the prostate via disruption of the normal endocrine environment, resulting in suppression of androgen production, or may elicit direct biological effects by targeting estrogen receptors (ERs) within the prostate itself. Initially, we implied local estrogen synthesis in the prostate gland by demonstrating aromatase expression in human prostate tissues. In further studies we described direct estrogen actions during prostate development using neonatal rodent organ culture experiments, which showed estrogens to be growth inhibitory. In order to further decipher direct estrogen actions mediated via the specific receptors ERα and ERβ, estrogen receptor knock out (ERKO) mice and ER-specific agonists were utilized in similar organ culture experiments. Taken together with the differential expression of ER subtypes in the prostate, we believe that estrogen signaling mechanisms can be selectively modulated to control prostate growth. Finally, our laboratory has used the estrogen-deficient aromatase knockout (ArKO) mice to demonstrate a role for estrogens in regulation of prostate growth. An imbalance of androgens and estrogens results in perturbed prostate size and pathology, but not necessarily malignant changes. The involvement of estrogens in prostate diseases, both benign and malignant, is an ongoing focus of our studies.

In summary, this laboratory has made a seminal contribution to the prostate field by demonstrating unequivocally that estrogens significantly impact on prostate development and disease.

018

THE ROLE OF ESTROGENS IN THE TESTIS AND THE MALE BRAIN – LESSONS FROM KNOCKOUT MICE

W. Boon , R. A. Hill , K. M. Robertson , M. E.E. Jones , E. R. Simpson

Prince Henry's Institute Of Medical Research, Clayton, Vic, Australia

Aromatase cytochrome P450 (Cyp19) catalyses the synthesis of C18 estrogens from C19 androgens. This enzyme is encoded by the *Cyp19* gene which is disrupted in the aromatase knockout (ArKO) mouse (1). Hence, this ArKO mouse model is an estrogen deficient animal model. Analyses of ArKO mouse phenotypes have provided information on the roles played by estrogens in the male animal, particularly in the testis and male brain. The male ArKO mice display progressive infertility with age. No differences were observed in the testicular weight in 12-14 week-old animals. At this age, there were no differences in the numbers of Sertoli cells, spermatogonia, spermatocytes and elongated spermatids between the wild type (WT) and ArKO mice. These ArKO animals have normal sperm

concentrations but decreased motility. By 1 year of age, both round and elongated spermatids were significant decreased in the ArKO mice (2) with concomitant decrease in testis weight, sperm concentration and motility. In addition, the male ArKO mice began to show impaired sexual behaviour around 14 week of age (3). This could be correlated to the decrease in sypnatic vesicles in the male ArKO medial preoptic area (MPO). The MPO has been reported to be involved in the regulation of male sexual behaviour and aggression. In the 1 yo ArKO, there was a significant decrease in numbers of dopaminergic neurons in the MPO and arcuate nucleus. These could be a direct result of apoptosis of dopaminergic neurons in these areas, as shown by immunohistochemical double-labelling with antibodies against active caspase-3 and tyrosine hydroxylase (4). In summary, estrogen plays an important role in the survival of spermatids in the testis and dopaminergic neurons in the hypothalamus of male mouse - both integral to the male fertility.

(1) Fisher et al (1998) PNAS USA. 95:6965-70.
(2) Robertson et al (1999) PNAS USA.; 96:7986-91
(3) Robertson et al (2001) J Androl. 22:825-30.
(4) Hill et al(2004) Molecular and Cellular Neuroscience. In press.

019

ESTROGENS, ANDROGENS AND CARDIOVASCULAR DISEASE IN MAN.

D. S. Celermajer

Cardiology, University Of Sydney, Camperdown, NSW, Australia

There is a striking gender difference in the incidence of atherosclerosis, with men having earlier onset and more extensive disease than women.

Although much initial information implicated a protective effect of estrogens, this hypothesis has not been well supported by recent large clinical trial results.

Thus more recent attention has focussed on the role of Androgens in atherogenesis. We have recently demonstrated that androgens accelerate 2 important early atherogenic events, monocyte adhesion to endothelial cells and macrophage lipid loading, and elucidated the underlying mechanisms.

In this talk, the role of androgens in atherogenesis will be presented.

020

ESTROGEN RECEPTORS IN BONE: INSIGHTS FROM KNOCKOUT MICE

N. A. Sims

Department of Medicine At St. Vincent's Hospital, The University Of Melbourne, Fitzroy, Vic, Australia

A need for estrogen in the growth and maintenance of bone mass in men and women is well established, but the roles of the two estradiol receptors (ERα and ERβ), and non-ER-mediated mechanisms remain poorly understood. A number of groups have studied bone phenotypes of ER knockout mice, but the phenotypes observed are not identical with each other, or with a lack of ER signalling in humans. To determine the relative contributions of each ER and non-ER-mediated mechanisms, gonadectomy and estradiol treatment have also been studied in these knockouts, yet the results have been complicated by differences in the knockouts used. In all female double ER KOs studied, bone mass was consistently low, and was not reduced further by ovariectomy, indicating that any protective effect of estrogen is lost by deletion of both receptors. In full double ER KOs, estradiol treatment did not alter either bone mass or ovarian size, indicating that both effects require the presence of one or both ERs. The use of gonadectomy and estradiol treatment in the absence of either ERα or ERβ reveal that, in female mice, while ERα is the major receptor mediating osteoprotective effects of estradiol, ERβ is also able to mediate a bone-protective effect of estradiol. In males, however, it appears that only ERα regulates of bone response to estradiol. This sexual dimorphism in the roles of these receptors may explain why ERα mutations have been noted only in men, since in women the activity of ERβ may compensate. Furthermore, the effects of testosterone treatment on the skeleton are unaffected by the absence of either ER, confirming that aromatisation is not required for an anabolic effect of testosterone in bone. This is supported by recent data in the congenitally androgen deficient (hpg) mouse, where testosterone and dihydrotestosterone treatment exhibit identical anabolic effects. In conclusion estrogen maintains bone mass largely through its action on the ERα, particularly in males, but in females the ERβ is able to play a minor role.

021

IDENTIFICATION AND STUDY OF GENES IMPORTANT FOR FETAL GERM CELL BIOLOGY IN MICE

P. Koopman [1,2], C. Browne [1,2], A. Jackson [1,2], K. Ewen [1,2,3], R. J. Aitken [1,3], J. Van Den Bergen [1,4], P. Western [1,4], A. Sinclair [1,4]

[1] ARC Centre of Excellence in Biotechnology and Development, Australia
[2] Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia
[3] School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia
[4] Murdoch Children's Research Institute & Dept. of Paediatrics, The University of Melbourne, Parkville, VIC, Australia

We are using a multi-pronged approach to discovering genes and proteins that regulate the allocation, proliferation, migration, differentiation, and apoptosis of primordial germ cells (PGCs) in the developing mouse embryo. First, we are using suppression PCR and microarray screening methods to identify genes whose expression is restricted to, or enriched in, gonads of a specific sex or developmental stage. In this way several genes were identified whose expression is restricted to germ

cells. Second, we are using a proteomic approach to identify important proteins and the genes that encode them. Protein expression profiles are being compared between different sexes and stages of mouse fetal gonad development. Data so far indicate that this method is a useful adjunct to transcriptional profiling, capable of identifying not only proteins that are differentially expressed, but also those that are differentially modified, for example by phosphorylation. Thirdly, in silico screening of mouse EST databases identified 23 new candidate genes whose expression appears to be limited to pluripotent cells and the germ line. Many of these genes are novel uncharacterised transcripts. Preliminary in situ expression analyses shows that 8 of these genes are indeed limited to the germ line and to pluripotent cells. These genes may have important functions in germ line specification and function. We are currently developing approaches, including inducible RNAi-based methods, for examining the function of these genes, initially in vitro, but also ultimately in vivo.

022

SPERMATOGONIAL STEM CELLS: FROM BASIC RESEARCH TO CLINICAL APPLICATIONS

S. Schlatt

Cell Biology and Physiology, University of Pittsburgh School of Medic, Pittsburgh, PA, United States

The testis contains undifferentiated spermatogonia and is therefore the only adult organ populated with proliferating germline cells. Whereas the biology of these cells is quite well understood in rodents, their modes of mitotic expansion and differentiation are poorly understood in primates. The existence of these cells offers clinically relevant options for preservation and restoration of male fertility. New approaches based on male germ cell transplantation and testicular tissue grafting can be applied to generate a limited number of sperm and could therefore be considered as important new avenues applicable to a variety of disciplines like animal conservation, genetic germline modification or restoration of fertility in oncological patients. In principle germ cell transplantation presents a removal of the stem cell from the donor's niche and a transfer into the niche of a recipient. Grafting can be considered as a transplantation of the stem cell in conjunction with its niche. Germ cell transplantation of human spermatogonia into mouse testes revealed that the stem cells survive and expand but are not able to differentiate and complete spermatogenesis. We have developed an approach to infuse germ cells into monkey and human testes and showed that germ cell transplantation is feasible as an autologous approach in primates. Furthermore, we applied germ cell transplantation in the monkey model mimicking a gonadal protection strategy for oncological patients. Ectopic xeno-grafting of testicular tissue was applied to generate fertile sperm from a variety of species. Newborn testicular tissue was grafted into the back skin of immunodeficient mice and developed up to qualitatively complete spermatogenesis. The rapid progress in the development of novel experimental strategies to generate sperm from cryopreserved spermatogonial stem cells or immature testicular tissue will lead to many new options for germline manipulation and fertility preservation.

023

DRIVERS OF GERM CELL DIFFERENTIATION

K. L. Loveland [1,2], C. Hogarth [1,2], A. Szczepny [1,2], C. Itman [1,2], A. Huebner [2,3], D. A. Jans [2,3]

[1] Institute of Reproduction and Development, Monash University, Clayton, VIC, Australia
[2] Australian Research Council Centre of Excellence in Biotechnology and Developmen, Australia
[3] Dept of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

Spermatogenesis requires progression of a self-renewing male germline stem cell population through a precisely timed and ordered developmental sequence to form spermatozoa. For several years, we have been investigating the functional impact of signals on this progression by members of the TGFβ superfamily, follicle stimulating hormone, and the bcl-2 family. For example, our lab and others have shown that activin A, bone morphogenetic protein (BMP) -4 and glial-derived neurotropic factor (GDNF) all modulate stem cell development and spermatogonial differentiation at the onset of spermatogenesis after birth. We are trying to understand how germ cells "interpret" to this plethora of competing signals to mediate maturation.

Progression through successive maturation states in response to such signals requires the movement of proteins, including transcription factors, into the nucleus to implement changes in gene transcription. We are investigating the concept that regulated transport of proteins into the nucleus is one mechanism that governs spermatogenic differentiation, and we have focused on analysis of the major class of nuclear transport factors, the importins (IMPs). A diverse family comprised of at least 6 different α forms and 20 different β forms in the mouse, the IMP proteins selectively bind a diverse range of cargo proteins and mediate their passage through the nuclear pore complex and into the nucleus. Our immunohistochemical and in situ hybridization studies have demonstrated developmentally regulated expression of many IMPs in germ cells, in the fetus, in the neonate and in the adult. This suggests that they function to transport cargo required for discrete stages of spermatogenesis. Our recent studies examined the mouse embryonic gonad around the time when specification to form either a testis or an ovary occurs. The IMPβ3 protein is present in both male and female germ cells, but the subcellular localization and expression patterns within these cell types is gender- and age-specific. We are currently exploring the functional significance of our observations.

024

POSTMEIOTIC GENE TRANSCRIPTS - SENSE, ANTISENSE, OR NONSENSE.

R. Ivell, R. J.K. Anand-Ivell

School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA, Australia

The mature spermatid is a highly specialized cell type whose structure and physiology have evolved to convey a haploid genome intact through a relatively inimical environment to find, interact with, and achieve fertilization of a mature female gamete, thus to produce a healthy diploid embryo. It is logical to assume that these highly specialist functions are the product of a unique set of genes or transcripts, expressed in late germ cell stages. It is equally logical to see the protein products of these genes as likely targets for a post-meiotic approach to male contraception. Indeed many such spermatocyte- and spermatid-specific transcripts have been identified by differential cloning approaches. Some

transcripts appear to represent novel sperm-specific genes, some represent sperm-specific alternative splice products, or alternatively initiated transcripts. However, for many such transcripts, there are features, which lend doubt to the notion that they are truly functional in the context of sperm physiology. Many transcripts derive from undefined, TATA-less promoters. Some gene products have no legible open reading frame. Some transcripts are even produced as antisense molecules. Some appear as functional transcripts, but are not translated. Some appear to be highly species-specific. Some appear to be functionally redundant, when tested in gene ablation experiments. The male gamete is under extreme selection pressure. It is therefore plausible that these apparently aberrant transcripts may have a function beyond that of conventionally generating physiologically relevant proteins, as in most somatic cells. This presentation reviews current ideas about the sperm transcriptome and presents various hypotheses to help us understand the mechanisms and purpose of post-meiotic gene expression

025

OVARIES: UP IN A POF OF SMOKE

A. N. Shelling , W. Smale , D. Prendergast , S. E. Harris , A. L. Chand , A. Ramachandran , K. Woad , I. M. Winship

Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

Premature ovarian failure (POF) or premature menopause is a common disorder, defined by the occurrence of menopause under the age of 40 years and is characterised by amenorrhoea, hypoestrogenism and elevated gonadotrophins. Worldwide it affects 1% of all women and occurs in 0.1% before the age of 30 years. The major problems associated with POF are the loss of fertility at an early age and the psychological problems associated with this. In addition there are the physiological effects of reduced oestrogen, which include an increased risk of osteoporosis. POF is a heterogeneous disorder and the cause of most cases is unknown. A significant proportion (20-30%) of women with POF have a genetic predisposition. Our primary goal is to identify genes involved in POF. In most cases, the menopause is due to the loss of follicles, and it stands to reason that suitable candidate genes for POF development would be genes that regulate the rate of follicle loss. We have identified two common gene mutations, a 769G>A transition in the inhibin alpha gene in approximately 5% of POF patients associated with POF at a very early age, and mutations in FOXL2 in approximately 5% of patients. FOXL2 is thought to act downstream of inhibin which suggests that other candidate genes may arise from the analysis of the activin signalling pathway. Functional studies will help us to understand more about the molecular basis of POF. Each mutation has been associated with less than 10% of POF cases and POF is likely to be caused by mutations at many different loci. It is hoped that determining the molecular basis of POF will lead to the development of genetic tests to predict the development of POF, and eventually lead to treatment that will return fertility to these women.

ENDOMETRIOSIS - LINKAGE, POSITIONAL CLONING AND GENOME WIDE ASSOCIATION

G. W. Montgomery [1], J. Wicks [1], Z. Z. Zhao [1], D. R. Nyholt [1], N. G. Martin [1], P. A.W. Rogers [2], S. A. Treloar [1]

[1] Genetic Epidemiology, QIMR, Herston, QLD, Australia

[2] Dept. Obstetrics & Gynaecology, Monash Medical Centre, Melbourne, VIC, Australia

Endometriosis is a complex disease which affects up to 10% of women in their reproductive years. Common symptoms include severe dysmenorrhea and pelvic pain. The disease is associated with subfertility and some malignancies. Genetic and environmental factors both influence endometriosis. The aim of our studies is to identify genetic variation contributing to endometriosis and define pathways leading to disease. We recruited a large cohort of affected sister pair (ASP) families where two sisters have had surgically confirmed disease and conducted a 10 cM genome scan. The results of the linkage analysis identified one chromosomal region with significant linkage and one region of suggestive linkage. The regions implicated by these studies are generally of the order of 20-30 cM and include several hundred genes. Locating the gene or genes contributing to disease within the region is a challenging task. The best approach to the problem is association studies using a high density of SNP markers. The recent development of human SNP maps and high throughput SNP genotyping platforms makes this task easier. We have developed high throughput SNP typing at QIMR using the Sequenom MassARRAY platform. The method allows multiple SNP assays to be genotyped on the same sample in a single experiment. Throughput and genotyping costs depend critically on this level of multiplexing and we routinely genotype 6-8 SNPs in a single assay. We are using bioinformatics and functional approaches to develop a priority list of genes to screen early in the project. SNP markers in these genes are being genotyped using the MassARRAY platform to search for genes contributing to endometriosis. In the future, genome wide association studies with our families may locate additional genes contributing to endometriosis.

027

MAPPING NOVEL BREAST CANCER SUSCEPTIBILITY GENES BY LINKAGE ANALYSIS OF AUSTRALIAN MULTIPLE CASE KINDREDS

G. J. Mann [1], G. M. Pupo [1], B. Newman [2], D. J. Venter [3], J. L. Hopper [4], G. Chenevix-Trench [5]

[1] Westmead Millennium Institute, University of Sydney, Westmead, NSW, Australia
[2] School of Public Health, Queensland University of Technology, Kelvin Grove, QLD, Australia
[3] Murdoch Children's Research Institute, Parkville, VIC, Australia
[4] Centre for Genetic Epidemiology, University of Melbourne, Carlton, VIC, Australia
[5] Queensland Institute for Medical Research, Herston, QLD, Australia
[6] Kathleen Cuningham Consortium for Research into Familial Breast Cancer, Australia

We have been using the resources of the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab) and of the Australian Breast Cancer Family Study (ABCFS) to identify kindreds suitable for mapping high penetrance breast cancer susceptibility loci other than BRCA1 and BRCA2. A 10cM genome-wide search was carried out in 40 families in which BRCA1 and BRCA2 mutations had been excluded with high probability. The highest LOD score under heterogeneity (HLOD) was 2.16 (non-parametric LOD 1.83, p=0.04) at the 11p telomere; several other regions with HLODs = 1.5-2.0 also merited investigation using fine mapping but have so far neither been confirmed or rejected by these analyses. Subsets based on age of onset and presence of other cancers correlated to some extent with particular linkage peaks and several regions (notably 2q and 13q) corresponded to areas of suggestive linkage reported recently in more limited studies of other cohorts. A large collaborative analysis of these data together with those from similar studies undertaken by members of the international Breast Cancer Linkage Consortium (BCLC) is under way. It is therefore likely that further major breast cancer susceptibility loci will be localized in the near future. The complementarity of these studies with genetic association, candidate gene and tumour-based approaches will be discussed.

028

NEUROBIOLOGY OF OVARIAN STEROIDS: "IT AIN'T JUST SEX ANYMORE"

B. S. McEwen

Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinolog, The Rockefeller University, New York, United States

Gonadal hormones have been known since 1849 to affect behavior, and ovarian steroids are now known to have effects on the brain throughout the lifespan, beginning during gestation and continuing on into senescence. Besides affecting the hypothalamus, amygdala and other brain areas related to reproduction and maternal behavior, ovarian steroids have widespread effects throughout the brain, on catecholaminergic neurons and serotonergic pathways and the basal forebrain cholinergic system, as well as the basal ganglia, hippocampus, cerebral cortex, spinal cord and cerebellum. Because of the widespread influences on many brain regions, ovarian steroids have measurable effects on cognition, emotion, motor control, pain and attention. They also have neuroprotective effects, eg, in models of ischemia. Cell nuclear receptors for estradiol 17□□□E) and progesterone (P) are found in greatest density in hypothalamus, preoptic area and amygdala, with much lower densities in other brain regions. E and P effects in these and other brain regions may be mediated by non-genomic

effects via a host of signaling pathways. Indeed, E and P receptors are present outside of the cell nucleus in dendrites, synapses and glial cell processes in brain regions not known for high densities of nuclear E and P receptors. The actions of E and P via non-genomic and genomic pathways will be illustrated for one process, namely, the regulation of synapse turnover in the hippocampus, a brain region important for spatial, declarative, episodic and contextual memory. E and P effects will also be noted in relation to cognition, aging and neuroprotection and the effects of synthetic progestins versus natural progesterone will be noted in relation to post-menopausal hormone therapy. Gonadal hormones play an important role in programming the brain during early development and gender differences in hippocampal responses to gonadal hormones will be noted as an illustration of the interaction between developmental and activational effects. Gender differences in the brain response to stress will also be noted. Aromatization of testosterone to estradiol plays an important role in sexual differentation and adult brain function, and recent evidence suggests an even broader role involving the local generation of estrogens from cholesterol in brain in response to neural activity and brain damage. Finally, other hormones play important and synergistic roles with gonadal steroids in a broad array of behaviors. This will be noted for oxytocin in terms of parenting and affiliative behavior and stress responsiveness and for prolactin in relation to anxiety.

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029

REPRODUCTIVE AGING AND THE HUMAN HYPOTHALAMUS

N. E. Rance

Pathology, University of Arizona College of Medicine, Tucson, Arizona, United States

The goal of our laboratory has been to characterize the cellular and molecular events that occur in the human central nervous system in response to the ovarian failure of menopause. The initial studies were based on the observations by Sheehan and Kovacs, who described hypertrophied neurons in the hypothalamic infundibular (arcuate) nucleus in postmenopausal women. We confirmed that hypertrophy does occur in a subpopulation of neurons expressing neurokinin B, substance P and estrogen receptor alpha gene transcripts. Subsequent studies have shown elevated levels of neurokinin B, substance P, GnRH and NPY mRNA in the hypothalamus of older women as well as decreased numbers of neurons expressing proopiomelanocortin (POMC) mRNA. Stereologic studies revealed no loss of neurons in the infundibular nucleus of older women, thus these changes could not be explained by cell death. To determine if loss of ovarian steroids could produce comparable changes in hypothalamic gene expression in young primates, we have recently measured the effects of ovariectomy on hypothalamic NKB, GnRH, NPY and POMC mRNA in young cynomolgus monkeys. Serum leptin and body weight were also measured because aging in women is accompanied by significant changes in energy homeostasis. We found that ovariectomy of young cynomolgus monkeys simulated the changes in NKB and GnRH gene expression in the postmenopausal human hypothalamus but the parameters related to energy balance (NPY and POMC gene expression, serum leptin and body weights) were unchanged. These data provide evidence that the increased NKB and GnRH gene expression in older women is a result of the ovarian failure of menopause. In contrast, the changes in NPY and POMC gene expression may be due to factors unrelated to the withdrawal of gonadal steroids. Our studies of the primate hypothalamus have suggested that NKB neurons function in the hypothalamic regulation of reproduction.

030

MECHANISMS OF ESTROGEN−INDUCED PLASTICITY IN GNRH NEURONS

A. E. Herbison

Centre for Neuroendocrinology, Dept. Physiology, University of Otago, Dunedin, New Zealand

Plasticity in gonadotropin-releasing hormone (GnRH) neuron activity through the oestrous cycle depends critically on estrogen feedback. However, the mechanisms used by estrogen to regulate the biosynthetic and secretory behaviour of these neurons remain unclear. The recent demonstration that GnRH neurons express estrogen receptor β (ERβ) has provided the impetus to re-examine the role of direct estrogen actions upon these cells. To investigate the potential physiological role of ERβ expressed by GnRH neurons, and ERα expressed by afferents to these cells, we have examined estrogen negative and positive feedback actions upon GnRH neurons in ERα and ERβ knockout mice *in vivo*. Estrogen positive feedback to generate the GnRH/LH surge was found to depend entirely upon ERα and involve cells located in the AVPV. Similarly, estrogen's negative feedback influence upon GnRH mRNA expression was found to be dependent upon ERα. So what role, if any, might ERβ have in GnRH neurons? We recently demonstrated that estrogen exerts rapid, non-genomic actions upon intracellular signaling cascades impacting upon the phosphorylation of CREB within GnRH neurons. The rapid estrogen effect was found to be direct upon the GnRH neuron and, in this instance, absent in only the ERβKO mouse. This suggests that ERβ expressed by GnRH neurons is involved in mediating rapid, non-classical estrogen actions upon these cells. Whether these non-genomic actions fluctuate during the estrous cycle is unknown at present. Together, these studies suggest the involvement of both ERα and ERβ receptors in different modalities of estrogenic signaling to GnRH neurons.

031

ESTROGEN FEEDBACK REGULATION TO GONADOTROPIN RELEASING HORMONE (GNRH) CELLS INVOLVES SERIAL AND CONVERGING PATHWAYS; STUDIES IN THE SHEEP

I. J. Clarke , S. Pompolo , O. Ischenko , A. Periera

Neuroendocrinology, Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Estrogen powerfully regulates the synthesis and secretion of GnRH, although the GnRH cells do not possess high levels of estrogen receptors. Using a combination of anterograde and retrograde tracing in the brain of the ewe, we have defined major pathways to the preoptic area (POA), that originate from the brainstem and the hypothalamus. These projections rarely provide direct input to the GnRH cells, suggesting inter-neuronal systems. Further tracing studies have identified the local circuits within the POA. Tracing has also been combined with immunohistochemistry, to define the types of cells that form the pathways. Estrogen responsiveness has been mapped, determining which cells possess estrogen receptors and also show a Fos response to estrogen challenge. We are now able to define pathways for estrogen feedback to GnRH cells. With the aid of confocal microscopy, we have

examined putative synaptic inputs to the GnRH cells. The resultant data show that glutamatergic cells provide the greatest level of input to GnRH cells (approximately 80% of cells contacted). Inputs from cells that produce gamma amino-butyric acid (GABA) are provided to approximately 30% of GnRH cells. GABAergic inputs are higher in the non-breeding season, compared to the breeding season, indicating plasticity. Inputs from noradrenergic systems and noradrenergic systems do not alter with season, suggesting that the predominant change is in negative regulators of GnRH cells.

032

GROWTH HORMONE DEFICIENCY – THE CLINICAL SPECTRUM AND TREATMENT OPTIONS

P. E. Clayton

Endocrine Science Research Group, University Of Manchester, Manchester, United Kingdom

Inadequate GH secretion has many aetiologies ranging from congenital hypopituitarism due to pituitary transcription factor defects, through isolated genetic GH deficiency (GHD) to low GH secretion in a short, slowly growing but otherwise normal child. Acquired disease or exogenous insults, such as CNS irradiation, affecting the hypothalamic-pituitary (hp) axis can also result in a spectrum of hormone deficiency from isolated GHD to panhypopituitarism. Although severe GHD is relatively straightforward to recognise both clinically, auxologically and biochemically, the diagnosis of moderate isolated GHD presents the clinician with considerable difficulty. Markers of the GHD state, such IGF-I, IGFBP-3 and ALS, provide some help in diagnosis, as does MR imaging of the hp axis, but in some cases a trial of GH treatment may also be part of the diagnostic process. The spectrum of the *functional* GH deficient state can be widened further by considering those rare conditions associated with abnormalities of GH activity; these include abnormal GH molecules that fail to activate the GH receptor (GHR), classical GH insensitivity (Laron syndrome) due to GHR defects, abnormal GH signalling pathways and IGF-I mutations. All of the disorders that adversely influence GH secretion or action have widened our understanding of the GH-IGF axis, but as yet we are often left to rely on results from poorly standardised GH provocation tests to make diagnoses and treatment decisions. The child with clearly defined inadequate GH secretion needs lifelong GH replacement to normalise growth and metabolism, including bone density, body composition and lipids. As many aetiologies for childhood GHD involve hypothalamic dysfunction, GH releasing hormone can be used but offers little advantage over GH. More recently GH has been given as a 2 weekly depot injection, but this is not proved superior to daily GH. For those with growth failure and normal releasable pools of GH, GH secretagogues, which can be orally active, have been used only in clinical trials. The challenge within this field remains the diagnosis of the range of GH deficient states, and most importantly the identification of the child who will benefit long-term from GH treatment.

033

THE ROLE OF IGF-1 IN CLINICAL PRACTICE.

B. R. King

Paediatrics, John Hunter Children's Hospital, Newcastle, NSW, Australia

Laron syndrome or growth hormone insensitivity syndrome (GHIS) is a very rare condition caused by abnormalities of the growth hormone receptor. It is characterised by a dysmorphic phenotype, short

stature, obesity, hypoglycaemia, elevated growth hormone (GH) levels, low insulin like growth factor 1 (IGF-1) levels and low insulin like growth factor binding protein (IGFBP3) levels. Human recombinant IGF-1 is currently considered the gold standard treatment for patients with GHIS. We describe two patients with GHIS, one patient who has completed treatment with IGF-1 and a second patient who is starting on IGF-1/IGFBP3 combination therapy.

In patients with GHIS, IGF-1 therapy leads to significant improvements in final height and patient quality of life. However, treatment with IGF-1 does not completely reverse the effects of GHIS. When GH binds to the GH receptor it activate second messenger pathways. In hepatocytes, this results in the production of IGF-1. But the activated second messenger pathways also affect cell metabolism, function and structure. It appears that GH and IGF-1 are synergistic in their action on protein and glucose metabolism. Because GHIS is rare, commercial production of IGF-1is not financially viable. This has made the treatment of GHIS patients difficult because therapy is only available through research programs. Researchers have been exploring the role of IGF-1 therapy for other conditions such as diabetes mellitus and catabolic states.

034

OPTIMIZING GROWTH AND HORMONE REPLACEMENT IN TURNER SYNDROME

M. Zacharin

Department Of Endocrinology & Diabetes, Royal Children Hospital, Parkville, Vic, Australia

Turner's syndrome affects 1/2500 live born phenotypic females. Timing of diagnosis varies from a pre-natal presentation on ultrasound through neonatal features of dysmorphism, childhood onset of short stature, to a late diagnosis with delayed or arrested puberty or irregular menses. Its significance lies in physical disabilities of short stature, cardio-vascular problems, renal anomalies and the consequences of ovarian dysfunction, with later questions of osteoporosis, lipid abnormalities,auto immune disorders and management of infertility and pregnancy. Cardiovascular disorders remain the largest single cause of mortality. SHOX gene haploinsufficiency accounts for the Turner phenotype. Growth deceleration commences in early childhood.Paediatric growth hormone treatment results in improved final height outcome by 5-6 cm, related to age at start of GH and family heights. Introduction of HRT should use low dose to allow peer group appropriate physical development, with rate of dosage change determined by age, growth potential and other treatments such as growth hormone. Later dosage increases require attention to emotional and psychological state and timing of menarche. A management plan must be discussed with the patient for comprehension, compliance and comfort. Patients with Turner's syndrome can now expect pregnancy induction with donor ova as a significant possibility. A high miscarriage rate (due to uterine factors) has been reported but the outcome is similar to that for other infertile couples and may change with earlier oestrogen treatment. Aortic dissection remains a major risk. Long term HRT may improve lipid profile, endothelial function, liver function and maintenance of bone quality.

037

AMP-ACTIVATED PROTEIN KINASE: LINKING ADIPOCYTOKINE SIGNALING TO METABOLIC CONTROL

D. I. Stapleton

St. Vincent's Institute, Fitzroy, Vic, Australia

AMP-activated protein kinase (AMPK) is a powerful metabolic regulator that functions as a critical focal point for whole body and cellular mechanisms maintaining energy homeostasis. AMPK is a heterotrimer with multiple subunit isoforms encoded by 7 genes and requires phosphorylation on the catalytic subunit by LKB1, a serine/threonine kinase, mutated in Peutz-Jeghers syndrome for activity. AMPK coordinates energy metabolism is response to the adipocyte-derived hormones leptin, adiponectin and resistin. In addition, AMPK activates fatty acid beta-oxidation, glycolysis, glucose transport, and stimulates food intake, whilst inhibiting fatty acid, triglyceride and cholesterol synthesis. In this way, AMPK functions as a critical focal point for whole body and cellular mechanisms maintaining energy homeostasis. AMPK also serves to control both gene transcription and protein synthesis, adapting the body to new regimes of energy supply and demand. The importance of AMPK as a key regulator has been further highlighted by the discovery of mutations in the AMPK ? subunits in pigs that cause glycogen storage disease as well as in humans that affect the electrical properties of the heart. Several lines of evidence link AMPK to glycogen metabolism, however the recent identification of a glycogen-binding domain in AMPK provides a molecular relationship between AMPK and glycogen. The role of the glycogen-binding domain in AMPK signalling together with recent advances will be presented.

038

INTERLEUKIN-6 AND INSULIN SENSITIVITY: FRIEND OR FOE?

M. Febbraio , A. L. Carey , A. G. Holmes , J. L. Mesa , M. Chan , N. Hiscock , M. J. Watt

Skeletal Muscle Research Laboratory, School of Medical Sciences, RMIT, Melbourne, Vic, Australia

Interleukin (IL)-6 is a pleiotropic cytokine produced by many organs including adipose tissue giving rise to it being labelled an "adipokine". However, recent work has demonstrated that this cytokine is produced within muscle cells (1), whereby it is subsequently released into the circulation where it can increase both lipolysis (2) and hepatic glucose production (3), providing substrate for contracting myocytes. In this case, IL-6 has been identified as the first "myokine", providing evidence that skeletal muscle, like adipose tissue, can be termed an endocrine organ. It is somewhat paradoxical that IL-6 is produced in marked quantities during muscle contraction but is associated with insulin resistance, since habitual exercise is associated not with insulin resistance, but enhanced insulin sensitivity (for review see 4). Recent work from our laboratory has focussed on the effect of both acute and chronic IL-6 administration on glucose tolerance, insulin sensitivity and intracellular signalling. We have found that in insulin sensitive cells such as L6 myotubes and 3T3-L1 adipocytes, acute IL-6 administration increases insulin stimulated glucose uptake by about 20% an effect that can be partially abolished by treating cells with the PI3-kinase inhibitor wortmannin and/or the p38 MAP kinase inhibitor SB203580. Consistent with these results, we have shown in L6 myotubes treated with insulin and IL-6, that phosphorylation of Akt and p38 MAPK are enhanced when compared with treatment of cells with insulin alone. Moreover, we have shown that glucose infusion rate during a

hyperinsulinemic euglycemic clamp in humans is increased by 20% when IL-6 is administered. Taken together, our data suggest that acute IL-6 enhances insulin sensitivity. To determine the effect of chronic IL-6 administration on insulin action, we have recently conducted experiments where rats were treated with IL-6 by min-osmotic pump (chronic IL-6 treatment) or by twice daily injections (phasic IL-6 treatment). Irrespective of mode of delivery, IL-6 treatment markedly improved glucose tolerance, without major effects on insulin sensitivity or insulin signalling in muscle tissue. We are currently conducting experiments to elucidate a mechanism for these results.

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039

TNFα

J. B. Prins [1, 2]

[1] ***Diabetes And Endocrinology, Princess Alexandra Hospital, Woolloongabba, Australia***
[2] ***Centre for Diabetes and Endocrine Research, University of Queensland, Woolloongabba, QLD, Australia***

TNF has had a chequered history as an important adipokine. It was initially proposed as a major biochemical link between obesity and T2DM, based on data indicating a positive correlation between circulating TNF, insulin resitance and obesity. This proposal was supported by animal studies in which anti-TNF strategies proved beneifical in insulin resistant models. When similar studies in humans with T2DM failed to show benefit, the role of TNF in human disease was questioned. More recent studies have further elucidated the complexity of the TNF system and its role in the metabolic syndrome. It is clear that TNF has roles in a variety of componenets of the metabolic sydrome, ranging from influencing insulin production and sensitivity through regulation of adiposity to regulation of end-organ damage. Evidence now suggests a role for TNF production from adipose tissue immune cells in addition to the fat cells, and the communication wetween these cell types is under active investigation. The recognition that many facets of the metabolic sydrome have an inflammatory basis has led to greater interest in local, rather than systemic, TNF concentrations and effects. For example, evidence supports roles for TNF in vascular disease and non-alcoholic fatty liver disease. Finally, the relationships between TNF and adiponectin, leptin and the PPAR systems has received much research effort recently, and data suggests that some of the benefiical effects of adiponectin and the TZDs may be due to modulation of the TNF system in target tissues. These recent studies and data have led to a renaissance of interest in TNF, and the presentation will address old and new data within an historical context.

040

OVARIAN RESEARCH IN THE POSTGENOMIC ERA: ANALYSES OF GDF-9 AND OTHER PARACRINE SIGNALING PATHWAYS

A. J. Hsueh

Stanford University, Stanford., United States

Recent publication of the genomic sequences for human and multiple model organisms allows the elucidation of the evolutionary origins of human genes. Because of the coevolution of polypeptide ligands and their cognate receptors, analysis of human genomic sequences allows one to predict the pairing of these elements. Based on a genomic approach, we identified the known BMPRII and ALK5 as the receptors for GDF-9 (Biol Reprod 2002; Mol Endocrinol 2004) and elucidated downstream signaling Smad molecules in ovarian follicles. We also identified LGR7 and LGR8 as receptors for relaxin and INSL3, respectively (Science 2002; JBC 2002). Based on the known production of INLS3 by testicular Leydig cells and ovarian theca cells, we investigated the expression of the INSL3 receptor, LGR8, in male and female gonads. Of interest, LGR8 expression was found exclusively in oocytes in the ovary and male germ cells in the testis. We further found that LH stimulates INSL3 transcripts in ovarian theca and testicular Leydig cells. INSL3, in turn, binds LGR8 expressed in germ cells to initiate meiotic progression of arrested oocytes in preovulatory follicles *in vitro* and *in vivo* and to suppress male germ cell apoptosis *in vivo* (Kawamura et al PNAS 2004). In contrast to its stimulation of cAMP production by somatic cells, INSL3 interacts with germ cells to activate the inhibitory G protein, thus leading to decreases in cAMP production. Our data demonstrate the importance of the INSL3-LGR8 paracrine system in mediating gonadotropin actions in gonads. We have set up an ovarian gene database (Ovarian Kaleidoscope database at http://ovary.stanford.edu) in which more than 1,600 ovarian gene pages can be accessed online and searched by gene name, function, mutation phenotype, expression pattern, cellular location, and other parameters. Database analysis of polypeptide ligand and receptor genes provides a functional genomic paradigm for the identification of novel ligands and receptors in the ovary. To assist ovarian researchers in the analyses of their DNA array datasets, a Microarray Data Interpreter has been set up in the OKdb to facilitate searches and comparisons of known and novel genes in the ovary.

041

ACTIVIN A: FROM REPRODUCTIVE FACTOR TO INFLAMMATORY CYTOKINE

D. J. Phillips , K. L. Jones , A. E. O'Connor , K. M. Wilson , S. Patella , K. Sebire , D. M. De Kretser

Monash Institute of Reprod. & Dev., Monash University, Clayton, VIC, Australia

Activin A was originally isolated and characterised as a reproductive feedback regulator of follicle-stimulating hormone. While potent paracrine networks involving activin and its binding protein, follistatin, are present in the gonad and pituitary, recent focus has been on emerging roles in a number of other systems, such as erythropoiesis, neuronal survival, embryonic development and inflammatory processes. The latter relatively new property was first suggested by us when follistatin in the

circulation was elevated in sheep undergoing surgical trauma. We have since focussed on a model of acute inflammatory challenge using the bacterial cell wall component, lipopolysaccharide (LPS) or endotoxin. This has highlighted that the release of activin into the bloodstream occurs extremely rapidly, within about 50 minutes. The response appears to be biphasic and precedes or is at least coincident with the release of a number of key pro-inflammatory cytokines, such as tumour necrosis factor α and interleukin-6. The mechanisms of this release are still being delineated, but it is fever- and prostaglandin-independent, and largely unaffected by blocking other key cytokine responses. Nevertheless, it is directly downstream of the LPS receptor and its activation pathway. Importantly, activin's property as an inflammatory cytokine appears to be borne out in a number of clinical inflammatory syndromes such as septicemia, suggesting that it is a hitherto undescribed component of the organism's innate immune response to infection.

042

THE ROLE OF TGF-BETA IN NORMAL AND PATHOLOGICAL LENS DEVELOPMENT

J. W. McAvoy [1], F. J. Lovicu [2], R. U. De Iongh [3]

[1] Save Sight Institute, University Of Sydney, Sydney, Australia
[2] Anatomy & Histology, University of Sydney, Sydney, Australia
[3] Anatomy & Cell Biology, University of Melbourne, Parkville, VIC, Australia

How the lens develops its highly ordered architecture and growth patterns is a major question in developmental biology. During embryogenesis, cells in the anterior and posterior segments of the lens vesicle, differentiate into the epithelial and fibre cells, respectively. Our research has aimed to identify the molecules and mechanisms that regulate the divergent fates of lens cells. We have studied the roles of various growth factors in regulating lens cell fates using rat lens epithelial explant cultures and transgenic and mutant mouse models. Our research has shown that members of the FGF growth factor family are key initiators of lens fibre differentiation in mammals and there is now compelling evidence that a gradient of FGF in the eye controls lens polarity and growth patterns. Recent evidence also supports a role for TGF-beta signalling in this process and indicates that a cascade of growth factor signalling may be required for normal fibre differentiation. Less is known about the anterior segment; however, our recent studies point to an important role for the Wnt growth factor family in epithelial differentiation. Growth factor signalling can also cause pathological changes; e.g. TGF-beta can destabilise the normal epithelial phenotype and induce aberrant growth and differentiation that mimics the epithelial-mesenchymal transition characteristic of some forms of cataract. These studies highlight the importance of growth factor signalling in regulating the ordered growth and differentiation of the lens. It is also clear that the bioavailabity of some growth factors needs to be tightly regulated so that they act in the appropriate cellular compartment. Some cataracts may be a consequence of disturbed growth factor, particularly TGF-beta, regulatory mechanisms.

043

ROLE OF TGFBETA IN ADRENAL STEROIDOGENESIS BEFORE BIRTH

C. L. Coulter

Centre for the Early Origins of Adult Health, University Of Adelaide, Adelaide, SA, Australia

During mammalian development there are periods when the fetal adrenal is either relatively refractory or increasingly sensitive to trophic stimulation. This pattern of regulation of adrenal growth and function ensures that the fetal lungs, liver, brain and kidney are exposed in a programmed temporal sequence to the genomic actions of circulating glucocorticoids. A range of studies in the rat and sheep have also demonstrated that exposure to excess glucocorticoids at inappropriate times in fetal life inhibits fetal growth and permanently reprograms the development of the cardiovascular and metabolic systems resulting in postnatal hypertension, abnormal hepatic glucose production and poor glucose tolerance In most mammalian species, there are therefore a range of mechanisms which protect the fetus from exposure to glucocorticoids of either maternal or fetal origin at inappropriate times in gestation. The factors which act to maintain periods of adrenal quiescence are not known. There is evidence that intra-adrenal Transforming growth factor beta 1 (TGFb1) is an inhibitor of adrenocortical steroidogenesis in the adult. In recent studies, we have demonstrated that expression of TGFb1, is high in the fetal sheep adrenal at around 100 d gestation and that adrenal TGFb1 expression then falls with increasing gestational age and is lowest immediately after birth. Following the activation of adrenal cytochrome P450 C17 (CYP17), there is an inverse relationship between adrenal TGFb1 and CYP17 expression and TGFb1 may therefore play a novel inhibitory role in the regulation of adrenal steroidogenesis during mid and late gestation. Whilst functional activation of the fetal adrenal is dependent on the fetal hypothalamo-pituitary axis, adrenal TGFb1 mRNA expression is not altered by disconnection of the fetal hypothalamus and pituitary in late gestation. It therefore appears unlikely that TGFb1 mRNA expression is regulated directly by either bioactive ACTH or cortisol in late gestation. The mechanism by which TGFb1 expression is upregulated in mid-gestation, remains to be determined.

044

THE SHORT-TERM EFFECTS OF HIGH DOSE TESTOSTERONE ON SLEEP, BREATHING AND FUNCTION IN OLDER MEN

P. Y. Liu [1,2], B. Yee [3], S. M. Wishart [2], M. Jimenez [2], D. G. Jung [3], R. R. Grunstein [3], D. J. Handelsman [2]

[1] Endocrine Research Unit, Mayo Clinic, Rochester, United States

[2] Andrology, ANZAC Research Institute, Sydney, NSW, Australia

[3] Sleep Medicine, Woolcock Institute of Medical Research, Sydney, NSW, Australia

Background Androgen therapy may precipitate obstructive sleep apnea in men. Despite increasing androgen use in older men, few studies have examined sleep and breathing. Randomised double-blind placebo-controlled studies examining effects of testosterone simultaneously on sleep, breathing and function in older men are not available. Methods Seventeen community-dwelling healthy men over the age of 60 were randomised to receive 3 injections of intramuscular testosterone esters at weekly intervals (500mg, 250mg and 250mg) or matching oil-based placebo, and then crossed-over to the other treatment after 8 weeks washout. Polysomnography, anthropometry and physical, mental and metabolic function were assessed at baseline and after each treatment period. Results Testosterone

treatment reduced total time slept (~1 hour), increased the duration of hypoxemia (~5 mins/night) and disrupted breathing during sleep (total and non-REM respiratory disturbance indices both increased by ~7 events/hour) (all $P<0.05$). Despite expected effects on body composition (increase in total and lean mass, reduction in fat mass, $P<0.05$, bioimpedance method), upper airway dimensions did not change (acoustic reflectometry). Neither were driving ability (computer simulation), physical activity (accelerometry, PASE), quality of life (SF36, FOSQ), mood (POMS), sleepiness (Epworth, Stanford scales) and insulin resistance (homeostasis model) changed by treatment. Conclusion Short-term administration of high-dose testosterone shortens sleep and worsens sleep apnea in older men, but did not alter physical, mental or metabolic function. These changes did not appear to be due to upper airway narrowing. Further study of longer-term lower-dose androgen therapy on sleep and breathing is needed to evaluate its safety in older men.

ORAL FREE COMMUNICATIONS

101

CLONING OF A NOVEL SRA-BINDING PROTEIN, SLIRP, THAT REGULATES ESTROGEN ACTION IN CANCER CELLS.

E. C. Hatchell [1,2], S. M. Colley [1,2], D. J. Beveridge [1,2], L. M. Stuart [1,2], R. B. Lanz [3], B. W. O'Malley [3], P. J. Leedman [1,2]

[1] Centre for Medical Research, WAIMR, University of WA, Perth, WA, Australia
[2] School of Medicine and Pharmacology, University of WA, Perth, WA, Australia
[3] Division of Molecular & Cellular Biology, Baylor College of Medicine, Houston, Texas, United States

SRA (Steroid Receptor RNA Activator)[1], the only known RNA coactivator, plays an important role in transactivation of the estrogen receptor. SRA expression is aberrant in many human breast tumours suggesting a potential role in pathogenesis. The structure of SRA is complex, containing multiple stem-loops, some of which contribute to transactivation capacity[2]. We have previously identified a family of SRA-binding proteins that target a specific stem-loop[3]. The aim of this work was to identify additional SRA-binding proteins targetting other SRA stem-loops critical for coactivator activity. Using yeast three-hybrid screening with STR7 (a key SRA stem loop), we cloned a novel protein SLIRP (SRA stem-Loop Interacting RNA-binding Protein), from a human breast cancer cell library. SLIRP encodes a 109 amino acid protein, containing an RNA Recognition Motif (RRM) spanning 80 amino acids. SLIRP mRNA is widely expressed in both human cancer cell lines and normal human tissue. Using an IP-RT-PCR assay we confirmed SLIRP is closely associated with SRA *in vivo*, and with gel-shift demonstrated binding of SLIRP to SRA STR7. SLIRP represses SRA-mediated transactivation of an estrogen-responsive reporter via its RRM. Mutation of the RRM abolishes the repression activity of SLIRP. Imaging studies using a SLIRP antibody reveal endogenous SLIRP localises to the mitochondria. Interestingly, SLIRP protein expression is regulated by estrogen (E_2). SLIRP shares high homology with another recently discovered SRA-binding protein SHARP[4], which contains an RRM and represses SRA coactivation. In gel-shift studies, SHARP binds STR7, raising the possibility that SHARP and SLIRP compete for binding. In summary, SLIRP is a novel SRA-binding protein that is a repressor of E_2 activity, contains a conserved RRM domain, is localised predominantly in the mitochondria, and is regulated by endogenous E_2 levels. SLIRP may well have a central role in the regulation of E_2-mediated gene expression in hormone dependent cancer, and modulation of cell growth.

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102

IMPACT OF CHRONIC LOW DOSE GLUCOCORTICOIDS ON BODY COMPOSITION AND PROTEIN METABOLISM

M. G. Burt , G. Johannsson , J. Gibney , K. C. Leung , K. K.Y. Ho

Pituitary Research Unit, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

Protein metabolism occurs almost exclusively within lean body mass (LBM). High dose glucocorticoids (GC) acutely increase protein breakdown and oxidation, which is irreversible, but do not affect synthesis (1). How chronic GC impact on protein metabolism is uncertain. Studies in Cushing's syndrome have yielded contradictory results, as the confounding effects of reduced LBM were not considered. The aim was to assess the effect of chronic low dose GC, typical of therapeutic use in inflammatory conditions, on body composition and protein metabolism. Whole body protein turnover was studied in 12 subjects (11F) with inactive polymyalgia rheumatica receiving chronic (>12 months) prednisone (2-10mg/day) and 12 age-matched normal subjects (11F) using a 3-hour primed constant infusion of 1-[13C] leucine, from which rates of protein breakdown, oxidation and synthesis were estimated. Body cell mass (BCM) was derived by subtracting extracellular water (ECW) (measured by bromide dilution) from LBM, which together with fat mass (FM) and bone mineral content (BMC) were measured using DXA. Percentage FM, BMC and ECW were similar in the two groups; and % BCM was not significantly reduced in GC users (34.5±2.0% vs 38.4±1.3%, p=0.13). BCM was significantly correlated with rates of protein breakdown (r=0.84, p=0.0006) and synthesis (r=0.79, p=0.002) in normal subjects but not GC users. Rates of protein breakdown (Δ16±7mg/min, p=0.015) and synthesis (Δ17±5mg/min, p=0.01), corrected for BCM, were greater in GC users, while oxidation was similar (Δ1.5±2.0mg/min, p=0.69). Protein breakdown (r=0.37, p=0.07) and synthesis (r=0.50, p=0.01) both correlated with prednisone dose. In summary, during chronic GC use, protein breakdown and synthesis are increased proportionately while protein oxidation is similar to normal subjects. This contrasts with the enhanced oxidation seen with acute GC. We conclude that normalization of irreversible oxidative loss of body protein may represent a critical adaptive response that protects against further loss of body protein. (Supported by St Vincent's Clinic Foundation, Pfizer and NHMRC of Australia)

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103

THE ROLE OF NOVEL SRA-BINDING PROTEINS IN NUCLEAR RECEPTOR PATHWAY CO-REGULATION.

A. D. Redfern [1] , D. Beveridge [1] , R. Lanz [2] , L. Stuart [1] , B. O'Malley [2] , P. J. Leedman [1]

[1] Medicine and Pharmacology, University of Western Australia, Perth City, WA, Australia

[2] Division of Cell and Molecular Biology, Baylor College of Medicine, Houston, Texas, United States

Nuclear receptor (NR)-mediated activation of target genes involves a diverse range of cofactors. SRA (steroid receptor RNA co-activator) (1), the only described RNA co-regulator, modulates transactivation for Type 1 NRs. To delineate the role of SRA in breast cancer we identified by yeast 3-hybrid screening three members of a family of double stranded RNA binding proteins - PACT, TARBP and PKR – which interact with SRA. Each binds SRA 'in vitro' and 'in vivo' as well as modifying SRA-mediated co-activation of estrogen action in transfections. The aim of this work was

to examine the functional role of both wild type and selectively mutated SRA-binding proteins across a broader range of NRs and to evaluate the combinatorial effects of the group on NR transactivation. PKR was shown to be a powerful co-repressor of signaling for estrogen, glucocorticoid and thyroid hormone receptors, an effect requiring both the RNA binding and kinase functions of the protein. TARBP inhibits this PKR-mediated repression in keeping with TARBP's known PKR-suppressive capabilities. Contrary to expectations, however, PACT, a known activator of PKR in translation inhibition pathways, has a stimulatory effect on signaling for a range of NRs, an effect that was synergistic with SRA. Mutational studies suggested PACT has a dual function, an RNA-binding independent PKR activation role and a powerful RNA-binding dependent stimulatory role which predominates with the wild-type molecule. Chromatin immunoprecipitation (ChIP) assays indicate that PKR associates with the estrogen response element of the endogenous cathepsin D gene in an estrogen-dependent manner in breast cancer cells. Additionally, PKR accentuates the inhibitory effect of anti-estrogens ICI182780 and 4hydroxy-tamoxifen whereas over-expression of PACT, particularly in combination with SRA, can remove estrogen signaling inhibition. Taken together, these results support an important physiological role for these proteins in estrogen signaling and suggest that altered expression could lead to anti-estrogen resistance.

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104

LACK OF ESTROGEN IN MALE MICE MAY BE LINKED TO OBSESSIVE COMPULSIVE DISORDER

R. A. Hill [1], M. E.E. Jones [1], E. R. Simpson [1], W. Boon [1]

[1] Breast Cancer Group, Prince Henry's Institute of Medical Rese, Melbourne, VIC, Australia
[2] Biochemistry, Monash University, Melbourne, VIC, Australia

Aromatase is the enzyme that converts androgens to estrogens. The aromatase knockout (ArKO) mouse lacks a functional aromatase, hence is an estrogen deficient model. Previously, we reported that female ArKO mice exhibit apoptosis in the frontal cortex, whilst the 1yo male ArKO show apoptosis in the arcuate nucleus and medial pre-optic area (MPOA). The MPOA amongst other functions has been reported to be involved in regulating grooming and running wheel behaviours, which have both been labelled as obsessive compulsive disorder (OCD) traits. Running wheel activity in the ArKO and WT mice was recorded by a wodometer, i.e. a running wheel with a counter. Grooming activity was recorded according to a ranking system: shaved face scored 3, shaved snout 2, clipped whiskers 1 and 0 to mice with all their hair intact. In the 1yo female ArKO mice, we found a decrease in running wheel activity, which is consistent with previous reports. In both the 6mth ($p = 0.08$) and 1yo ($p = 0.002$) male ArKO, we found an increase in running wheel activity compared to WT counterparts. In addition, grooming behaviour is increased in both 6mth ($p = 0.04$) and 1yo male ArKO mice ($p = 0.06$) compared to WT controls, whilst no significant difference in grooming patterns was observed in 6mth and 1yo female ArKO as compared to WT. Grooming and running wheel activity are increased in the 1yo male but not female ArKO, which correlate to the disruption we observed in the MPOA of the male but not female ArKO. Several reports have linked estrogen to OCD behaviours. Therefore, we propose that lack of estrogen in the male animal results in medial pre-optic area disruption, which may be associated with development of OCD traits such as running wheel activity and grooming.

105

LGR7-TRUNCATE IS AN ALTERNATIVE MRNA SPLICING VARIANT OF THE RELAXIN RECEPTOR, LGR7, AND IS A RELAXIN ANTAGONIST *IN VITRO*

D. J. Scott [1], E. Hopkins [1], S. Layfield [1], S. Sudo [2], A. J.W. Hsueh [2], G. W. Tregear [1], R. A.D. Bathgate [1]

[1] Howard Florey Institute, The University of Melbourne, Parkville, VIC, Australia
[2] Department of Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, California, United States

Relaxin-1 is a heterodimeric hormone primarily produced by the pregnant corpus luteum. Relaxin breaks down components of the extracellular matrix and acts as a modulator of tissue remodelling, a stimulator of cervical ripening and is implicated in prolongation of myometrial quiescence during pregnancy. The relaxin-1 receptor is leucine-rich repeat-containing GPCR 7 (LGR7). LGR7 has a large extra-cellular domain containing leucine-rich repeats and an N-terminal Low Density Lipoprotein Class-A-module (LDLa module). An LGR7 mutant lacking the LDLa module binds relaxin-1 but is unable to stimulate cAMP signaling, demonstrating a role for the LDLa module in mediating ligand directed receptor activation. While cloning the mouse ortholog of LGR7, an exon 4-deleted mRNA splicing variant was identified. This variant, named LGR7-Truncate, has a premature stop codon resulting in a transcript encoding a protein comprising primarily LGR7's LDLa module. Employing real-time PCR, LGR7-Truncate mRNA expression was detected to be equal to that of LGR7 in late pregnant mouse uterus, however LGR7-Truncate mRNA was not detected in the cerebral cortex, where LGR7 expression is highest. Hence the hypothesis that LGR7-Truncate is an endogenous mediator of LGR7 function was devised. LGR7-Truncate was cloned into the mammalian expression vector pcDNA3.1/Zeo. 293-T cells co-transfected with LGR7-Truncate and LGR7 exhibited reduced cAMP accumulation upon relaxin treatment compared to cells co-transfected with LGR7 and empty vector. LGR7-Truncate, affinity purified from the media of transfected cells, was able to inhibit cAMP accumulation in relaxin treated stably transfected LGR7 cells. These experiments revealed LGR7-Truncate as the first relaxin antagonist to be identified. LGR7-Truncate orthologous transcripts were subsequently identified in rat, pig and human, suggesting LGR7-Truncate is an evolutionarily conserved autocrine modulator of relaxin activity. LGR7-Truncate represents a novel mode of GPCR regulation and if active *in vivo* may be an endogenous regulator of relaxin's effects on female reproductive function.

106

MECHANISM OF PHOTOPROTECTION BY VITAMIN D COMPOUNDS.

K. M. Dixon [1], S. S. Deo [1], R. Gupta [1], M. Slater [1], G. M. Halliday [2], V. E. Reeve [3], R. S. Mason [1]

[1] Department of Physiology and Institute for Biomedical Research, The University of Sydney, Sydney, NSW, Australia
[2] Department of Medicine (Dermatology), The University of Sydney, Sydney, NSW, Australia
[3] Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia

Vitamin D is produced by the exposure of 7-dehydrocholesterol in the skin to UV irradiation (UVR), and can be further converted in the skin to the biologically active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). Exposure to UVR also produces mutagenic photoproducts, including cyclobutane pyrimidine dimers (CPDs). We exposed cultured human skin cells and Skh:HR-1 mice to UVR in the presence of $1,25(OH)_2D_3$ or vehicle. The presence of $1,25(OH)_2D_3$ significantly reduced CPDs in UV-irradiated keratinocytes, melanocytes and fibroblasts in a dose-dependent manner from 0.5h after UVR, with similar results in UV-irradiated mouse skin, assessed 24h after irradiation. UVR also results in high nuclear levels of the p53 tumour suppressor protein in skin cells, which in turn leads to cell cycle arrest, facilitating DNA repair, though p53 can also cause apoptosis of cells that are too damaged for adequate DNA repair. Treatment with $1,25(OH)_2D_3$ increased nuclear p53 protein expression in UV-irradiated keratinocytes and melanocytes several fold. Nitric oxide (NO) products, which can impair DNA repair, also increased following UVR. $1,25(OH)_2D_3$ reduced nitrite in the cultures to a similar extent to the known NO synthase inhibitor, aminoguanidine. Moreover, aminoguanidine also reduced CPD in keratinocytes, measured 3h after irradiation. We propose that the increased survival of skin cells after UVR in the presence of $1,25(OH)_2D_3$, previously reported, is due to enhanced nuclear p53 expression together with reduced NO products, which both increase DNA repair. These results indicate that the local vitamin D system in skin may contribute to endogenous photoprotection and suggest the possibility of enhancement of this process by topical application of vitamin D compounds.

107

AMINO ACID SUBSTITUTIONS IN IGFBP-2 ALTER BINDING TO EXTRACELLULAR MATRIX COMPONENTS AND AFFECTS NEURONAL CELLS RESPONSES TO GROWTH AND MIGRATION.

V. C. Russo [1], B. S. Schutt [2], E. Andaloro [1], S. I. Ymer [1], A. Hoeflich [3], L. A. Bach [4], M. Ranke [2], G. A. Werther [1]

[1] Centre for Hormone Research, Murdoch Childrens Research Institute, Parkville, VIC, Australia
[2] University Children's Hospital, University of Tubingen, Tubingen, Germany
[3] IMAB Gene Center, Ludwig-Maximilians University, Munich, Germany
[4] Dept. of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC, Australia

Modulation of IGF action by the IGFBPs occurs in the pericellular space involving interactions with ECM components and cell surface. We have previously demonstrated that IGFBP-2 binds to cell surface proteoglycans in brain explants and that IGFBP-2 is highly expressed during brain development and repair process. IGFBP-2 possess a putative heparin binding domain (HBD,

$_{218}$PKKLRP$_{223-}$) and an integrin binding motif ($_{265}$RGD$_{267}$), both potentially involved in cell surface association of IGFBP-2. We therefore aimed to alter hIGFBP-2 HBD and RGD sequences by mutagenesis, and determine the biological effect of these mutations. The HBD of hIGFBP2, ($_{218}$PKKLRP$_{223-}$) was mutated to ($_{218}$PNNLAP$_{223-}$) and the $_{265}$RGD$_{267}$ motif to $_{265}$RGE$_{267}$ by a PCR based mutagenesis. SK-N-SHEP neuroblastoma cells (IGFBP-2 negative) were stably transfected with either the HBD-, RGE-IGFBP-2 mutants or the WT-IGFBP-2. WT and mutants IGFBP-2 were purified by IGF-I affinity chromatography and quantified by an IGFBP-2 ELISA assay. The mutants and WT-IGFBP-2 maintained the ability to bind ^{125}I-IGF-I. Scatchard analysis revealed that the binding affinity of the IGFBP-2 mutants for IGF-I was only minimally affected compared to that of WT-IGFBP-2. The HBD-IGFBP-2 mutant showed dramatically reduced (~80%) binding to heparin and to ECM. Binding of the RGD-IGFBP-2 mutant to heparin or ECM was comparable to the WT-IGFBP-2. Cellular growth was potently reduced (~2-3 fold over 96h) in SHEP cell clones over-expressing the HBD-IGFBP-2 mutant compared to the that of SHEP cell clones expressing equal amount of WT-IGFBP-2. Similarly, in a in vitro cell adhesion and invasion assays, the SHEP cells over-expressing the HBD-IGFBP-2 mutant show reduced adhesion to ECM components (~2 fold) and dramatic reduction (~70%) of invasion. The RGD mutant did not affect growth, adhesion or invasion of the SHEP cells. These findings demonstrate, for the first time, that the predicted HBD at $_{218}$PKKLRP$_{223}$ in IGFBP-2 is involved in binding to heparin and ECM components and that the ablation of these interactions dramatically affects cellular growth, adhesion and invasion and therefore interactions with the extracellular environment. Our findings thus suggest a potential key role for the HBD in IGFBP-2 in brain development and repair processes of the nervous system.

108

ROLE OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-5 TERNARY COMPLEXES IN INSULIN-LIKE GROWTH FACTOR-I BIOAVAILABILITY

S. M. Firth [1,2], F. K. McDougall [1,2], P. J.D. Delhanty [1,2], R. C. Baxter [1,2]

[1] Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, NSW, Australia
[2] University of Sydney, NSW, Australia

Insulin-like growth factor (IGF) binding proteins, IGFBP-3 and IGFBP-5, are main regulators of the insulin-like activities of IGFs in circulation by sequestering the IGFs in ternary complexes with acid-labile subunit (ALS). This study examined the role of IGFBP-5 ternary complexes in the regulation of IGF-I bioavailability. Male Wistar rats were administered with an intravenous bolus injection of recombinant human (rh)IGF-I, rhIGFBP-5, or rhIGF-I + rhIGFBP-5 (binary complex) via a jugular vein cannula, and blood samples were taken at various time points post injection. Coadministration of IGF-I + IGFBP-5 prevented IGF-I-induced hypoglycaemia seen with IGF-I alone. The clearance rate of coadministered IGF-I + IGFBP-5 from the circulation was significantly slower than when IGF-I ($p<0.05$) or IGFBP-5 ($p<0.0001$) was administered alone. This was due to the rapid sequestration, within 2 minutes, of IGF-I and IGFBP-5 into ternary complexes, more of which was retained in the circulation compared to the binary form. This suggests that the clearance rate of the ternary complex will have an impact on IGF-I bioavailability. To determine the contribution of ALS to the clearance rate of the ternary complex, we have conducted similar infusion studies of IGFBP-5 binary or ternary complexes or rhALS alone in male GH-deficient spontaneous dwarf rats that have undetectable levels of circulating ALS. rhALS was cleared significantly faster when administered alone than when

administered as IGFBP-5 ternary complexes ($p<0.0001$). Surprisingly, there was no significant difference in the clearance profiles of IGFBP-5 when administered as binary or ternary complexes, both of which were cleared more slowly than IGFBP-5 alone ($p<0.01$). These results suggest that IGF-I is the determining factor in the clearance of both binary and ternary IGFBP-5 complexes. Taken together with our recent study which showed that IGFs sequestered in IGFBP-5 ternary complexes are more retarded in transendothelial transport than either unbound or binary complexed IGFs (1), IGFBP-5 ternary complexes serve to prolong the circulating half-life of IGF and prevent IGF egress from the circulation.

(1) Payet LD, Firth SM and Baxter RC (2004) The role of the acid-labile subunit in regulating insulin-li

109

INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) INHIBITS INSULIN STIMULATED GLUCOSE UPTAKE IN ADIPOCYTES.

S. S.Y. Chan [1,2], S. M. Twigg [2], R. C. Baxter [1,2]

[1] kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, NSW, Australia
[2] Discipline of Medicine, University of Sydney, sydney, NSW, Australia

IGFBP-3 is the major serum IGFBP which binds insulin-like growth factors (IGFs). At a cellular level, IGFBP-3 regulates bioactivity of IGFs and it also exerts IGF-independent actions. Transgenic mice over-expressing IGFBP-3 have insulin resistance and hyperglycaemia with reduced glucose uptake into skeletal muscle and adipose tissue. We aimed to examine the effects of IGFBP-3 on cultured mouse 3T3-L1 adipocytes and to determine the mechanisms by which IGFBP-3 may affect insulin resistance in fat cells. Consistent with the IGFBP-3 transgenic model, IGFBP-3 treatment of adipocytes reduced insulin stimulated glucose uptake. A dose response study showed that 50ng/mL of IGFBP-3 in serum free medium reduced insulin stimulated glucose uptake maximally by 40.7% ($p=0.016$). The effect was time dependent with IGFBP-3 (50ng/mL) causing a decrease in insulin stimulated glucose uptake by 36% ($p<0.001$) at 90m and a maximal decrease of 44% ($p<0.001$) at 24h. IGFBP-3 mutated to abolish its nuclear translocation and retinoid X receptor binding retained its inhibitory activity, indicating that the inhibitory process occurs at an extranuclear site. The inhibitory activity of IGFBP-3 was still present despite blockade of new protein synthesis by cycloheximide (10ug/mL). In contrast, another high affinity binding protein IGFBP-2 (up to 1ug/mL) had no effect on insulin stimulated glucose uptake suggesting that IGFBP-3 was not acting through sequestering IGF-1. Using a monoclonal antibody termed α IR3 which preferentially blocks the type 1 IGF-1 receptor (IGFR1), we showed that the effect of IGFBP-3 on reducing insulin stimulated glucose uptake was independent of the blockade of the IGFR1 and thus independent of IGF-1 acting through this receptor. Preliminary data using plasma membrane lawn studies have shown that IGFBP-3 inhibited the translocation of the glucose transporter GLUT-4 to the plasma cell membrane. Therefore, we examined the impact of IGFBP-3 on some key components of the classical insulin signalling pathway. We found that IGFBP-3 had no effect on insulin receptor phosphorylation in 3T3-L1 adipocytes. We are currently studying the effects of IGFBP-3 on serine and threonine phosphorylation of AKT. Further work will focus on the mechanisms by which IGFBP-3 leads to insulin resistance in adipocytes.

OESTROGEN AND SELECTIVE OESTROGEN RECEPTOR MODULATORS (SERM) EXERT DIVERGENT EFFECTS ON GROWTH HORMONE SIGNALLING THROUGH DIFFERENT MECHANISMS

K. Leung , J. Brce , N. Doyle , G. M. Leong , K. Sjogren , K. K.Y. Ho

Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, NSW, Australia

GH exerts biological effects through the JAK2/STAT5 pathway, which is terminated by the suppressors of cytokine signalling (SOCSs) and protein tyrosine phosphatases (SHP-1 and -2). We have recently reported that oestrogen inhibits GH signalling by suppressing JAK2 phosphorylation, an action mediated by SOCS-2[1]. The effects of SERMs on GH signalling are unknown. We compared the effects of 4-hydroxytamoxifen (4HT) and raloxifene (Ral) to 17β-oestradiol (E2) on the GH/JAK2/STAT5 cascade in HEK293 cells expressing human GHR and ERα. The cells were transfected with a luciferase reporter containing a STAT5 binding element, and treated with GH (500ng/ml) and E2 or SERMs (1, 10, 100nM) for 18h before measuring luciferase activity. JAK2 phosphorylation was assessed by Western blotting. Phosphatase activity was measured by immunoprecipitation of SHP-1 or -2 followed by colourimetic assay with synthetic phosphopeptide as substrate. GH alone stimulated STAT5 reporter activity by 3.6±0.7 fold (mean±SE; P<0.02). Co-treatment with E2 reduced the reporter activity dose-dependently to 62±4% of untreated control (P<0.02) at 100nM. In contrast, 4HT and Ral increased the activity maximally by 58±5% and 43±5% (P<0.02), respectively. GH-induced JAK2 phosphorylation was reduced by E2 to 57±4% of control, but increased by 4HT and Ral to 178±15% and 184±11%, respectively (P<0.01). Quantitative RT-PCR revealed that the SOCS-2 mRNA level was increased by E2 to 156±12% of control (P<0.05), but unaffected by SERMs. 4HT reduced the activity of SHP-1 and -2 in a time-dependent manner maximally to 62±7% and 61±6% of control, respectively (P<0.05). Ral decreased SHP-1 activity to 53±1% of control (P<0.05), but did not affect SHP-2. E2 did not affect phosphatase activity. In summary, SERMs enhanced the transcriptional activity of GH by promoting JAK2 phosphorylation, an effect involving phosphatases. We conclude that oestrogen and SERMs exert opposite effects on GH signalling through different mechanisms. (Supported by NHMRC and Eli Lilly Australia)

(1) Leung et al. (2003) PNAS 100:1016-21

IGF-I, IGFBP-3 AND ALS AS POTENTIAL MARKERS OF GROWTH HORMONE DOPING IN ELITE ATHLETES - EFFECTS OF DEMOGRAPHIC FACTORS AND SPORTING TYPE.

C. J. Howe [1], A. Nelson [2], T. Nguyen [2], K. Hardman [3], S. Meka [3], K. Leung [2], G. Trout [1], R. Baxter [3], D. Handelsman [4], M. Irie [5], R. Kazlauskas [1], K. Ho [2]

[1] Australian Sports Drug Testing Lab, Australian Government Analytical Laboratories, Pymble, NSW, Australia
[2] Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[3] Kolling Institute of Medical Research, St Leonards, NSW, Australia
[4] ANZAC Research Institute, Concord, NSW, Australia
[5] Toho Medical School, Toho University, Tokyo, Japan

IGF-I, IGFBP-3 and ALS are potential indirect markers of GH doping. The aim of this study was to determine the effects of demographic factors including ethnicity, age, sex and BMI on these markers, to establish reference ranges in a multi-ethnic population of elite athletes, and to investigate the effects of sport type on these markers. Blood samples were obtained on at least 3 successive occasions from 1098 elite athletes from 12 countries, including 587 Caucasians, 349 East Asians, 109 Africans and 53 from Oceanian and other groups. Athletes were assigned to eight categories of sporting type. Serum IGF-I, IGFBP-3 and ALS concentrations were measured by radioimmunoassay. Random-effects ANOVA was used to estimate the effects of ethnicity, age, BMI and sex. IGF-I, IGFBP-3 and ALS concentrations decreased with age, and each was positively correlated with BMI and was higher in females than in males. Ethnicity had a significant effect, with lower concentrations of all 3 markers in Africans compared to most other ethnic groups at $p<0.05$, except for IGF-I in females. IGFBP-3 and ALS were higher in Caucasian than in Africans by 15 – 20% (age- and weight-adjusted means, $p<0.0001$) in both males and females. Analysis of the effect of sports type using age- and weight-adjusted means, indicated higher IGF-I concentrations in power and power/endurance athletes and higher concentrations of IGFBP-3 and ALS in endurance and power sports athletes of both sexes.

In conclusion, demographic factors including ethnicity and sporting type are significant determinants of IGF, IGFBP-3 and ALS in elite athletes. These factors must be considered in defining reference ranges for GH doping in sports, and in the clinical utilisation of these markers. (Supported by the World Anti Doping Agency and Australian Government Anti-Doping Research Program).

112

IGF-I ADMINISTRATION DECREASES SEVERITY OF CHEMOTHERAPY-INDUCED MUCOSITIS VIA MODULATION OF EPITHELIAL CELL DYNAMICS.

J. C. Cool [1,2], J. L. Dyer [1], C. J. Xian [3], R. N. Butler [4], D. Tenikoff [1], M. S. Geier [1], R. Kamil [1], G. S. Howarth [1,2,4]

[1] Child Health Research Institute, Women's & Children's Hospital, Adelaide, SA, Australia
[2] Cooperative Research Centre for Tissue G, Women's & Children's Hospital, Adelaide, SA, Australia
[3] Department of Orthopaedic Surgery, Women's & Children's Hospital, Adelaide, SA, Australia
[4] Department of Gastroenterology, Women's & Children's Hospital, Adelaide, SA, Australia

Insulin-like growth factor-I (IGF-I) has been demonstrated to enhance mucosal repair following intestinal damage induced by chemotherapeutic agents (intestinal mucositis). However, the effect of prophylactically administered IGF-I on intestinal mucositis remains unclear. We investigated the effects of IGF-I pre-treatment on chemotherapy-induced mucositis in rats. Methods: Male Sprague Dawley rats (135 g) were treated for 7 days with vehicle or 4.3 mg/kg/day IGF-I. On day 7, rats received an intraperitoneal injection of 0 or 150 mg/kg 5-fluorouracil (5-FU). Rats were killed 48 hours later for assessment of intestinal damage and repair. Results: 5-FU decreased epithelial proliferation by 86%, concurrently increasing the incidence of apoptosis 87-fold, whilst decreasing small intestinal (SI) length by 14%, SI weight by 30% and total gut weight by 24%. 5-FU decreased villus height in the duodenum (23%), jejunum (20%) and ileum (30%) with crypt depths decreased by 31%, 27% and 33% in these gut regions. These effects were less profound in IGF-I pre-treated rats in which apoptosis was increased 48-fold, with SI length decreased by 7%, SI weight by 18% and total gut weight by 15% accompanied by decreases in villus height of 8% (duodenum), 14% (jejunum) and 21% (ileum), and crypt depth decreases of 23%, 16% and 17% for the same gut regions, compared to normal controls. Conclusion: We conclude that IGF-I treatment prior to chemotherapy partially attenuates features of intestinal mucositis.

113

IN VIVO REGULATION OF GENE EXPRESSION BY GROWTH HORMONE IN MUSCLE FROM HYPOPITUITARY MEN: A MICROARRAY STUDY

K. Sjogren [1,4], K. Leung [1], W. Kaplan [2], M. Gardiner-Garden [3], J. Gibney [1], J. Brce [1], K. K.Y. Ho [1]

[1] Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, NSW, Australia
[2] Peter Wills Centre for Bioinformatics, Garvan Institute of Medical Research, Sydney, NSW, Australia
[3] Cancer Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia
[4] RCEM, Department of Internal Medicine, Sahlgrenska University Hospital, Goteborg, Sweden

GH is a major regulator of muscle development. Little is known about GH genes involved in muscle growth and function. Using microarray analysis, we have studied GH-responsive genes in muscle biopsies obtained from six GH-deficient men before and after two weeks GH treatment (0.5 mg/day). Total RNA from each biopsy was extracted and analysed using the Affymetrix HG-U133A

GeneChips. Plasma samples were assayed for IGF-I, procollagen 1 and 3. GH treatment up-regulated 128 (107 known and 21 unknown) and down-regulated 8 genes (7 known and 1 unknown). Within the IGF system, GH induced a significant increase in IGF-I (2.2±0.4 fold; mean±SEM), but not IGFBP-3, 4, 5 or 6 expression. Among muscle proteins, GH significantly increased the expression of eleven collagen genes including COL1-α1, 1-α2 and 3-α1 (all >2.5 fold). In the signalling system, GH did not affect the GH receptor or JAK2, enhanced STAT1 (1.7±0.2 fold) but not the other STAT proteins, and significantly up-regulated SOCS-2 but not SOCS-1, 3 or CIS expression. Plasma levels of IGF-I, procollagen 1 and 3 increased significantly ($P<0.001$) with GH. In summary, GH stimulated muscle expression of IGF-I and collagens, and up-regulated STAT1 and SOCS-2 but not related family members. GH exerted IGF-I-mediated endocrine and paracrine effects on muscle, and transcriptionally regulated specific members of the STAT and SOCS family. These are the first human data on the effects of GH on gene expression in muscle. (Supported by NHMRC of Australia and Wennergren Foundation, Sweden)

114

DETECTION OF NOVEL BIOMARKERS OF HUMAN GROWTH HORMONE ADMINISTRATION USING SELDI-TOF MASS SPECTROMETRY

L. Chung [1], K. Ho [2], R. Kazlauskas [3], R. C. Baxter [1]

[1] Kolling Institute of Medical Research, University of Sydney, Royal North Shore, St Leonards, NSW, Australia

[2] Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

[3] Australian Sports Drug Testing Laborator, Pymble, NSW, Australia

The detection of human growth hormone (GH) administration by athletes poses unique analytical problems. Although the hormone itself is easily detected by highly sensitive assays, its short circulating half-life provides only a brief opportunity in which to detect the administrated hormone above the endogenous background level. To define new biomarkers of GH administration, we have investigated the use of surface-enhanced laser desorption/ionisation time-of-flight (SELDI-TOF) mass spectrometry that offers the advantages of speed and sensitivity. Sera from 60 subjects, sampled before and after 21 days of placebo or rhGH treatment (0.1 or 0.2 IU/kg.day; 20 subjects/group), were obtained from the double-blind, placebo-controlled GH administration trial conducted by the international GH2000 consortium (Dall et al, *J Clin Endocrinol Metab* 85:4193, 2000) and examined on protein chips with various chemical surfaces. Analysis of results to date suggests that the Cu^{2+}-IMAC30 chip binds a number of GH-regulated proteins which form a variety of ionized species with m/z (mass/charge) values in the 6000-16000 range, with either negative or positive GH-dependence. Some of these have apparent molecular masses consistent with monomeric and dimeric IGF-I isoforms. A total of 246 peaks common to the 120 profiles were identified, and peak intensities and m/z ratios were recorded (59,040 data values). Among these 246 peaks, several demonstrate statistically significant discrimination between placebo and GH treatment. Further identification of IGF-axis biomarkers of GH action is currently underway using antibody-capture protein chip arrays with IGF-I antiserum, which enables confirmation of protein identification. The high sensitivity and specificity achieved by using SELDI-TOF-MS show great potential for detection of human growth hormone administration. Supported by DCITA.

115

COLONIC INJURY AND REPAIR IN DIPEPTIDYL PEPTIDASE IV KNOCKOUT MICE WITH EXPERIMENTAL COLITIS INDUCED BY DEXTRAN SULPHATE SODIUM

M. S. Geier [1], C. A. Abbott [2], D. Tenikoff [1], R. Yazbek [2], G. W. McCaughan [3], G. S. Howarth [1]

[1] Child Health Research Institute, North Adelaide, Australia
[2] School of Biological Sciences, Flinders University, Adelaide, Australia
[3] A.W. Morrow Gastro.& Liver Centre, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Glucagon-like peptide-2 (GLP-2) is an intestinotrophic growth factor that enhances repair of damaged intestinal tissue. However it is rapidly inactivated by the serine protease, dipeptidyl peptidase IV (DPIV). In order to determine the role of DPIV in maintaining epithelial integrity we compared the development and resolution of experimental colitis in wild-type (WT) and DPIV gene knockout (DPIV-/-) mice and hypothesized that DPIV-/- mice would display an enhanced recovery from experimentally-induced colitis. WT and DPIV-/- mice consumed 2% dextran sulphate sodium (DSS) for 6 days, followed by a 15 day recovery period. Body weight and disease activity index (DAI) were monitored daily. Mice were killed at days 0, 3, 6, 9, 14 and 21 (n=6-8). Small intestine and colon were removed for assessment of villus height (VH), crypt depth (CD) and crypt area (CA). The epithelial cell proliferative labelling index (LI) was determined by proliferating cell nuclear antigen (PCNA) immunostaining. Statistical comparisons were by Student's T-test. No significant differences were detected between WT and DPIV-/- mice for any of the following parameters: small intestine and colon weight, rate of body weight loss DAI in the distal colon, CD at day 6 (WT: 144 ± 10 vs. DPIV-/-: 120 ± 20 mm), and day 14 (274 ± 36 vs. 315 ± 75 mm), CA at day 6 (44 ± 14 vs. 32 ± 19 %) and day 14 (49 ± 6 vs. 41 ± 12 %), LI at day 6 (0.04 ± 0.02 vs. 0.01 ± 0.01 %) and day 14 (0.29 ± 0.04 vs. 0.29 ± 0.04 %). We conclude that loss of DPIV activity does not increase resistance to experimental colitis, nor promote recovery, and speculate that the recently identified DPIV family members, DP8 and DP9 may be involved in GLP-2 degradation to provide targets for therapeutic intervention in colitis.

116

MARKED UPREGULATION OF COX-2 EXPRESSION IN RAT PLACENTA DURING LATE PREGNANCY

S. Hisheh, D. Hewitt, P. J. Mark, B. J. Waddell

Anatomy & Human Biology, The University of Western Australia, Crawley, WA, Australia

Placental prostaglandin (PG) synthesis and secretion impact on both fetal development and parturition. These PG levels are determined by local regulation of both PG synthesis and metabolism. PG synthesis is dependent, in part, on expression of the cyclooxygenase (Cox)-1 and -2 enzymes, both of which can be regulated by glucocorticoids. The rat placenta is unusual being comprised of two morphologically- and functionally-distinct regions of trophoblast tissue, the basal and labyrinth zones. In this study, we examined expression of Cox-2 mRNA and protein over the final third of

gestation, the period of maximal fetal and labyrinth zone growth. We also determined the effects of the exogenous glucocorticoid, dexamethasone, on placental Cox-2 expression. Previous studies had shown that while glucocorticoids generally suppress Cox-2 in various tissues, they stimulate Cox-2 expression in the placenta of the sheep and human. Dexamethasone acetate was administered to rats via drinking water (0.1 mg/ml) from day 15 to 22. Placentas were obtained at days 16 (untreated only) and 22 (untreated and dexamethasone-treated) and dissected into basal and labyrinth zones. Cox-2 mRNA expression was determined by realtime RT-PCR and Cox-2 protein levels by Western blot analysis. Cox-2 mRNA expression increased dramatically (20-fold, $p<0.001$) in the labyrinth zone between days 16 and 22, whereas Cox-2 protein expression showed a less marked increase ($p<0.01$). Expression of Cox-2 mRNA and protein in basal zone remained relatively stable over this same period. Treatment with dexamethasone increased Cox-2 mRNA twofold in basal zone ($p<0.01$) but had no effect on expression in labyrinth zone. These data demonstrate rat placental Cox-2 expression is highly dynamic and region-specific during late gestation, and is stimulated by glucocorticoids in the basal zone as previously reported for other species. The regulatory factors that stimulate a marked increase in Cox-2 expression specifically in the labyrinth zone remain to be elucidated.

117

PROTEOMIC ANALYSIS OF THE HUMAN AMNION AND CHORION AT NORMAL AND PRETERM LABOUR

R. Johnson [1], C. Mitchell [1], V. Murphy [2], Y. Wang [3], K. Akinsanya [3], R. Smith [1], T. Zakar [1]

[1] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

[2] Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, Newcastle, NSW, Australia

[3] Ferring Research Institute, San Diego, CA, United States

The fetal membranes (amnion and chorion laeve) produce a range of protein factors that may play essential roles in human labour. Surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS) is a powerful new technology predominantly used to detect low mass proteins. In this study we have characterized, by SELDI-TOF MS, the protein profiles of the human fetal membranes at term and preterm labour. Fetal membranes were collected after elective Cesarean section in subjects not in labour at term (TNL, n=5) and preterm (PNL, n=5), or following spontaneous term labour (TL, n=5) and following idiopathic preterm labour (PL, n=5). Proteins were extracted in urea/CHAPS buffer and applied onto strong anion exchange (SAX) and weak cation exchange (WCX) protein chips. Mass analysis was performed using SELDI-TOF MS and peaks were classified as significant with a $p<0.05$ (Mann-Whitney U Test) and a greater than two fold difference in the mean peak intensity. Using the WCX and SAX surfaces, we detected a total of 134 and 185 protein peaks, respectively, from the amnion and chorion. In both tissues, 12 protein peaks were significantly different between the TNL and TL groups. In the amnion, 5 peaks increased and 7 peaks decreased after term labour. In the chorion, 1 peak increased and 11 peaks decreased after term labour. After preterm labour 4 peaks were different in the amnion, all of them decreasing. In the chorion, after preterm labour, 21 peaks were different with 6 peaks increasing and 15 peaks decreasing. Notably, 36 of the 49 proteins that showed changes with term or preterm labour were smaller than 11kD. The differentially expressed peptides detected by the SELDI-TOF MS technology have potential

diagnostic and therapeutic significance. Further work is required to positively identify these peptides and characterize their functions in human parturition.

118

SEX SPECIFIC MECHANISMS OF HUMAN FETAL CORTISOL METABOLISM AND IMMUNITY

V. L. Clifton , V. E. Murphy , A. Osei-Kumah , N. Scott , W. B. Giles , P. G. Gibson , R. Smith

Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW, Australia

We have recently identified that when asthma, regardless of its severity, is not treated during pregnancy with inhaled steroids female fetal growth is significantly reduced. When the mother uses inhaled steroids female fetal growth is comparable to the control population. The male fetus appears unaffected by asthma or its treatment. These findings suggested that factors that regulate fetal growth are gender specific. We then questioned whether placental cortisol metabolism and pathways regulated by cortisol differ between the male and female fetus of normal pregnancies. Placental cortisol metabolism, cytokine and glucocorticoid receptor (GR) expression was assessed in placentae collected from normal term pregnancies. Data was analysed based on fetal sex. Female fetuses had significantly increased placental 11β -HSD2 activity (P=0.002), higher basal cytokine mRNA expression and increased GR and MR expression (P<0.05) relative to placentae from male fetuses. These findings suggested that the female fetus may respond differently to an inflammatory challenge due to increased cortisol metabolism. Cord blood cells from male and female fetuses were exposed to increasing concentrations of lipopolysaccharide (LPS) *in vitro* and tumour necrosis factor α(TNFα) measured. The female fetus had a more enhanced TNFα response to LPS than the male fetus. These data provide evidence there are different placental mechanism involved in fetal development based on gender. Furthermore the difference in immune function observed between the male and female fetus may explain the increased mortality rate of the male fetus in pregnancies complicated by chorionamnionitis.

119

PROGESTERONE RECEPTOR MRNA ABUNDANCE IN THE GESTATIONAL TISSUES OF THE GUINEA PIG

T. Welsh [1], T. Zakar [1,2], S. Mesiano [1]

[1] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

[2] Discipline of Obstetrics and Gynaecology, John Hunter Hospital, Newcastle, NSW, Australia

Progesterone withdrawal is a critical event leading to parturition onset. In most species, decreased circulating progesterone levels mediate progesterone withdrawal. However, parturition in women and guinea pigs is not accompanied by changes in circulating progesterone, and we have hypothesised that in these species parturition is initiated by a functional progesterone withdrawal due to changes in progesterone receptor (PR) expression in target tissues. The aim of this study was to characterise PR mRNA expression in guinea pig gestational tissues throughout late pregnancy. Amnion, visceral yolk sac (VYS; analogous to the human chorion), placenta and myo-endometrium were collected at 45d of pregnancy, at term (63-71d) and during labour. Real-time RT-PCR was performed to determine total PR mRNA abundance in these tissues. Guinea pig PR primers were based on conserved regions of PR

cDNA sequence from the human, mouse and rabbit. These primers yielded a 343 bp amplicon, which exhibited 92% identity with the corresponding human PR sequence. PR mRNA was predominantly located in guinea pig myo-endometrium, where levels were more than 2-fold higher at term than at 45d. During labour, levels increased a further 2.5-fold. PR mRNA was present in VYS and placenta at 45d and term, and expression did not differ in either tissue between these two stages. In contrast to the myo-endometrium, PR mRNA was undetectable in these tissues in labour. PR mRNA was not detected in guinea pig amnion. These data suggest that the myo-endometrium is the primary target tissue for progesterone in the pregnant guinea pig uterus. The physiological significance of low PR mRNA levels in the fetal membranes and placenta remains to be established. The ontogeny of PR mRNA in guinea pig gestational tissues is strikingly similar to findings in women, and therefore the guinea pig presents a promising model to study functional progesterone withdrawal at parturition.

120

MECHANISMS OF PROSTAGLANDIN SYNTHESIS REGULATION IN THE FETAL MEMBRANES

R. Johnson [1], C. Mitchell [1], W. Giles [1,2], W. Walters [1,2], T. Zakar [1,2]

[1] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

[2] Division of Obstetrics and Gynaecology, John Hunter Hospital, Newcastle, NSW, Australia

Prostaglandin endoperoxide synthase (PGHS)-2 expression increases in the human amnion and chorion with advancing gestation enhancing the capacity of the fetal membranes to produce labour-promoting prostaglandins. The mechanisms of PGHS-2 up-regulation are unknown. We have determined whether regulation occurs in vivo at the transcriptional or post-transcriptional stages of enzyme expression. Amnion and chorion (laeve) were collected from non-labouring women at 9-18 (ENL, n=18) and 28-36 gestational weeks (PNL, n=20), and also at term (38-41 weeks, TNL, n=26). PGHS-2 mRNA abundance was determined by quantitative real time RT-PCR (Q-RT-PCR). PGHS-2 gene activity was assessed by measuring the level of the PGHS-2 mRNA precursor, PGHS-2 hnRNA, by Q-RT-PCR. In the amnion, PGHS-2 mRNA abundance increased by late gestation with no further increase at term (ENL<PNL=TNL; $p<0.001$, Kruskal-Wallis ANOVA with multiple comparisons). PGHS gene activity exhibited the same pattern as the mRNA, and PGHS-2 hnRNA levels, but not gestational age grouping, significantly predicted (Robust Regression) PGHS-2 mRNA levels in individual tissues. Thus, PGHS-2 mRNA up-regulation in late gestation amnion was transcriptional. PGHS-2 mRNA abundance also increased in the chorion by late gestation (ENL<PNL=TNL), however, PGHS-2 gene activity remained the same throughout the gestational age groups. Moreover, gestational age grouping, in addition to PGHS-2 hnRNA level, was a significant predictor of PGHS-2 mRNA abundance in individual chorions, suggesting that in this tissue, the late-gestational up-regulation of PGHS-2 mRNA levels was predominantly post-transcriptional. We have detected significant correlation between amniotic and chorionic PGHS-2 gene activity in individual patients of the PNL and TNL, but not of the ENL groups suggesting that PGHS-2 transcription is coordinated in the two tissues before normal labour onset. PGHS-1 mRNA levels, measured by Q-RT-PCR, were high in both tissues in the ENL group and decreased dramatically by late pregnancy, implicating PGHS-1 specifically in the provision of prostaglandins during early gestation.

121

STEROID HORMONE REGULATION OF PLACENTAL CRH GENE EXPRESSION: A COMPLEX MIX OF MOLECULAR MECHANISMS

R. C. Nicholson

Mothers and Babies Reserach Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

Corticotrophin releasing hormone (CRH) is a 41-amino acid neuropeptide secreted by the paraventricular nucleus of the hypothalamus, and functions as the principal mediator of the HPA response to stress. CRH is also found in many tissues outside the central nervous system, in particular during human pregnancy this peptide is produced in large amounts by the placenta. Biosynthesis of placental CRH increases exponentially with advancing gestation, an increase mirrored by exponential increases in CRH concentration in maternal plasma. In the human, abnormally elevated maternal CRH levels during pregnancy are associated with pre-term delivery, whereas unusually low levels correlate with longer than normal gestations. Consequently, it has been suggested that placental CRH plays a key role in the timing of birth. A better understanding of how CRH production is controlled in the syncytiotrophoblast cells of the placenta is a prerequisite to gaining an insight into this CRH driven timing mechanism. A variety of endogenous factors, including steroid hormones are able to regulate placental CRH production. Glucocorticoids stimulate, whereas estrogen (E2) has a tonic inhibitory effect on CRH production in placental cells. Progesterone (P4) also decreases CRH production in the placenta, and some workers have proposed that this effect of P4 may occur through the glucocorticoid receptor (GR). We have conducted extensive molecular biological analyses of steroid hormone mediated regulation of the CRH promoter in placental cells. We have determined that a cAMP Regulatory Element (CRE) is crucial for regulation by glucocorticoids, E2 and P4. Glucocorticoids (via GR) stimulate, E2 (via ER a) represses, and P4 either represses (via PR-A or GR) or stimulates (via PR-B) CRH gene expression. These studies indicate that steroid hormone mediated action involves complex molecular mechanisms that modulate placental CRH production, thereby influencing the rate of rise of maternal plasma CRH concentrations and potentially the length of gestation.

REPEATED MATERNAL GLUCOCORTICOID TREATMENT REDUCES RENAL GLUCOCORTICOID RECEPTOR PROTEIN LEVELS IN FETAL SHEEP

A. J. Johnston [1], D. M. Sloboda [1,3], T. J. Moss [1,3], B. J. Waddell [2], J. P. Newnham [1,3]

[1] School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia

[2] School of Anatomy and Human Biology, The University of Western Australia, Perth, WA, Australia

[3] Women and Infants Research Foundation, Perth, WA, Australia

Background: The biological actions of glucocorticoids are mediated through activation of intracellular glucocorticoid receptors (GR). Functional GR are present in the ovine fetal kidney throughout gestation. In the kidney, glucocorticoids affect fluid and electrolyte balance. *Methods*: Pregnant ewes (n = 38) bearing single fetuses were injected intramuscularly (IM) with 150 mg of medroxyprogesterone acetate at 100 days of pregnancy (d; term is 150d). Ewes were then randomised to receive maternal IM injections of saline or betamethasone (0.5 mg/kg body weight) on 104, 111 and 118d. Groups of these animals were killed at 109d (n = 12), 116 (n = 12) or 146d (n = 14). Fetal body and kidney weights were measured and the right kidney was collected for molecular analysis. Renal GR protein levels were measured by Western blotting. Treatment groups were compared at each time point using unpaired t-tests. *Results*: Body weight and total kidney weight at 109d, after one dose of betamethasone at 104d, were not different between control and betamethasone fetuses. At 116d, after two doses of betamethasone, fetal body weight was reduced by 14% ($p = 0.022$) and total kidney weight reduced by 15% ($p = 0.061$). Fetuses delivered at 146d after three doses of betamethasone had body weights 30% lower than saline fetuses ($p = 0.003$) and total kidney weight was reduced by 28% ($p = 0.021$). GR protein levels were not different between saline and betamethasone-treated animals at 109d. Maternal betamethasone treatment reduced renal GR protein levels by 23% in 116d fetuses ($p = 0.079$) and by 21% in 146d fetal sheep ($p = 0.008$). *Conclusions*: Repeated administration of betamethasone to pregnant ewes reduces fetal body weight, total kidney weight and renal GR protein levels. A reduction in GR protein levels may decrease glucocorticoid hormone action in the kidney and affect tubular function.

123

PLACENTAL EXPRESSION OF PPARΔ, PPARΓ AND RXRA IN RAT PREGNANCY AND THE EFFECTS OF INCREASED GLUCOCORTICOID EXPOSURE.

D. P. Hewitt , P. J. Mark , B. J. Waddell

Anatomy and Human Biology, University of Western Australia, Nedlands, WA, Australia

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, acting as ligand-activated transcription factors primarily involved in lipid metabolism. Recent gene deletion studies indicate that PPARγ and PPARδ also play critical roles in mouse development, including effects on placental vascularization. In this study we investigated the spatial and temporal expression of PPARδ , PPARγ and the heterodimeric partner of PPARγ, RXRα , in the normal rat placenta and after glucocorticoid-induced fetal and placental growth restriction. PPARγ and RXRα expression was markedly higher in labyrinth compared with basal zone, at both e16 to e22. PPARδ expression was comparable in the two placental zones, but fell by 76% and 63% in the basal and labyrinth zones, respectively, from e16 to e22. Dexamethasone treatment (e13-22) reduced placental and fetal weights by 44% and 31% respectively, coinciding with a 37% fall in PPARγ expression in the labyrinth zone ($p<0.05$). However, there was no effect on the expression pattern PPARδ or RXRα with dexamethasone treatment. These data suggest that PPARγ and RXRα play important roles in the labyrinth zone during late pregnancy, possibly supporting vascular development. Moreover, it appears that glucocorticoid-inhibition of placental growth may be mediated, in part, via suppression of PPARγ specifically within the labyrinth zone. In contrast, the role of PPARδ may be diminished late in pregnancy, since its expression fell in both placental zones. Further studies are required to assess whether ligand activation of placental PPARs can influence placental growth and function.

124

TETRAHYDROGESTRINONE (THG) IS A POTENT ANDROGEN AND PROGESTIN

A. K. Death [1] , K. C.Y. McGrath [2] , T. Tsatralis [1] , R. Kazlauskas [3] , D. J. Handelsman [4]

[1] Heart Research Institute, Camperdown, NSW, Australia

[2] Discpline of Medicine, University of Sydney, Sydney, NSW, Australia

[3] Australian Sport Drug Testing Laboratory, AGAL, Pymble, NSW, Australia

[4] ANZAC Research Institute, Concord, NSW, Australia

Tetrahydrogestrinone (THG) is a novel steroid recently identified by a sports doping laboratory as an illicit agent sold to improve elite athletic performance. While its structure is closely related to gestrinone, a 19-nor progestin, and resembles that of trenbolone, a potent banned synthetic androgen, THG was never marketed, so no information on its hormonal properties are known. We therefore examined THG for steroidal bioactivity using yeast transformed with a steroid receptor-reporter system, comparing its bioactivity to other known androgens , nandrolone, 7alpha nandrolone (MENT), norbolethone, 5alpha-norbolethone, norethandrolone, , trenbolone, as well as THG's parent compound, gestrinone. Yeast were stably transformed with human androgen receptor (AR) or progesterone receptor A (PR) cDNA, together with a reporter plasmid containing a ß-galactosidase gene under the transcriptional control of an androgen (ARE) or progestin (PRE) reporter element.

Bioassays were established by culturing transformed yeast in the presence of the steroids over the range of $1.2X10^{-6}$ to $5.9X10^{-10}$M.The bioassay end-point was ß-galactosidase activity in yeast cell lysates. THG showed dose-dependent highly potent activation of AR activity with an EC_{50} of 0.29 nM compared with other steroids nandrolone (0.12 nM) norbolethone (0.3 nM), 5alpha-norbolethone (0.026 nM), gestrinone (0.59 nM), trenbolone (0.78 nM), norethandrolone (0.19 nM) and MENT (0.01 nM). THG also activated PR (EC_{50} 0.7 nM) with much higher potency than its parent steroid, gestrinone (EC_{50} 30 nM). We conclude that THG is a potent androgen and progestin. It shows similar potency to the comparator androgens, nandrolone, norbolethone, 5alpha-norbolethone and trenbolone. The discovery of this illicit designer androgen raises concern about the possibility of other novel androgens being produced from other marketed synthetic sex steroids.

125

DIFFERENTIAL REGULATION OF INHIBIN BINDING VIA BETAGLYCAN EXPRESSION IN SEVERAL MOUSE CELL LINES

P. G. Farnworth , Y. Wang , G. T. Ooi , J. K. Findlay

Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Inhibin A, a member of the transforming growth factor (TGF)-b superfamily, binds to mouse adrenocortical (AC), Leydig (TM3) and Sertoli (TM4) cell lines with high affinity via at least eight membrane protein species, two of which are forms of betaglycan. Inhibin A has been proposed to inhibit the actions of activin and BMP by sequestering their type II receptors in high affinity complexes with betaglycan [1]. We previously found that BMPs appear to counteract inhibin action in AC cells by selectively suppressing the expression of endogenous betaglycan, consequently reducing inhibin binding. In the present studies, we have examined how factors that stimulate betaglycan expression in other systems modify the binding of radiolabelled inhibin A at the surface of AC, TM3 and TM4 cells. AC, TM3 and TM4 cells were treated overnight with glucocorticoid, membrane-permeable cAMP analogue, or retinoic acid, after which the levels of betaglycan mRNA, corrected for GAPDH content, were measured using real-time RT-PCR, and [^{125}I]inhibin A binding was determined. Treatment of AC cell cultures with 8Br-cAMP (1 mM), glucocorticoid (RU28362, 100 nM) or retinoic acid (30 mM) increased betaglycan mRNA levels 120-150%, and increased subsequent inhibin A binding to 146±12, 132±13 and 125±18 % of control (mean±SD, *n*=6-12). The glucocorticoid and cAMP treatments also increased inhibin binding to TM3 and TM4 cells by similar amounts, but retinoic acid was less effective. Affinity labelled protein species of deduced sizes 115 and >170 kDa, consistent in size with betaglycan forms, were the primary target for stimulation by these agents, whereas species of 65 and 75 kDa were selectively increased by retinoic acid in the AC cells. In summary, glucocorticoids, retinoids, and hormones that stimulate cAMP production may increase the expression of betaglycan in inhibin target cells, increase their binding of inhibin, and thereby promote inhibin action. These studies confirm that betaglycan is a primary determinant of inhibin binding and action. The protein species other than betaglycan that are selectively up-regulated by retinoic acid in AC cells are yet to be identified.

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126

A REPOSITORY OF ENU MUTANT MOUSE LINES AND THEIR POTENTIAL FOR MALE FERTILITY RESEARCH

C. L. Kennedy [1,2], A. E. O'Connor [1], L. G. Sanchez-Partida [1,3], C. C. Goodnow [3], D. M. De Kretser [1,2], M. K. O'Bryan [1,2]

[1] Monash IRD, Monash University, Clayton, VIC, Australia
[2] ARC Centre of Excellence, Biotechnology and Development, Australia
[3] Australian Phenomics Facility, Australian National University, Canberra, ACT, Australia

1 in 25 western men are infertile and the causal factor is frequently unknown, although it is expected that many are genetic in origin. My project aims to identify genes critical to mouse spermatogenesis using ENU mutagenesis. A further aim was to develop a repository of mutant mice and data on their fertility parameters for use by the reproductive biology community. This research will aid the diagnosis and development of specific treatments for human infertility and the development of contraceptive agents. The potent mutagen *N-ethyl-N-nitrosourea* (ENU) was utilized to generate libraries of C57BL/6 mice with random point mutations throughout their genomes. A 3 generational breeding program produced mice that were homozygous for a number of mutations. I subsequently performed a number of large scale screens on 3rd generation males, identifying lines carrying recessive mutations specifically affecting male fertility. Thus far we have observed a wide range of abnormal testis phenotypes including Sertoli Cell only, hypospermatogenesis, meiosis arrest, abnormal sperm morphology and abnormal hormone levels. From these analyses a repository including all data and tissues collected from 1200 3rd generation male mice from 122 different lines has been developed and will become publicly available. This includes testis and epididymal histology and serum levels of FSH, LH, activin A and inhibin. Further, I have stored gDNA long term and cryopreserved sperm to enable regeneration of lines in the future. In addition, I have developed a high throughput mutation screening protocol for the detection of mutations within genes of interest using denaturing high performance liquid chromatography (DHPLC). Collectively, our repository and gene screening techniques can be used in conjunction with artificial reproductive technologies to generate mouse models reflective of human conditions and altered specific gene function.

127

AN A_6B_1-INTEGRIN/FOCAL ADHESION KINASE COMPLEX MAY REGULATE SPERMIATION AND SPERMIATION FAILURE

A. J. Beardsley [1,2], D. M. Robertson [1], L. O'Donnell [1]

[1] PHIMR, Prince Henry's Institute of Medical Rese, Clayton, VIC, Australia
[2] Deptartment Anatomy and Cell Biology, Monash University, Clayton, VIC, Australia

Spermiation is the final step of spermatogenesis (sperm production) where mature spermatids are released from the somatic Sertoli cells. Spermiation is hormone sensitive; testosterone (T) and FSH withdrawal causes a disruption to the disengagement of spermatids which are instead retained by Sertoli cells. The mechanisms involved with spermatid release and retention are not understood. We showed previously that an unknown adhesion junction containing β_1-integrin persisted on retained spermatids suggesting that a defect in this adhesion complex at disengagement may underlie spermiation failure. The aim of this study is to identify the α-integrin dimerised with β_1-integrin and investigate the role of phosphorylated FAK, a kinase that is involved with integrin-mediated cell adhesion, during spermiation and spermiation failure. Four adult Sprague-Dawley rats received T and

estradiol implants and FSH antibody for 7 days to suppress testicular T and FSH and induce spermiation failure. Using immunohistochemistry, α_6-integrin (but not α_4-integrin) and FAK-Tyr^{397} were localised on the Sertoli cell plasma membrane adjacent to mature spermatids. This localisation was observed until the point of spermatid release and remained on the Sertoli cell that surrounded retained spermatids after hormone suppression. A similar localisation has been previously observed with β_1-integrin, suggesting that all three form a complex at the site of disengagement. To look at the function of FAK-Tyr^{397}, comparative Western blot analysis is currently being undertaken on seminiferous tubules specific for spermiation from control and treated animals. Preliminary studies suggest that FAK-Tyr^{397} remains phosphorylated during spermiation failure, suggesting that FAK dephosphorylation may be important for the function of spermatid-associated adhesion complexes, as has been demonstrated in other adhesion systems. In conclusion, $\alpha_6\beta_1$-integrin/FAK-containing adhesion complexes are associated with spermatids during spermiation, and the function of such complexes are likely to be perturbed during spermiation failure.

128

RELAXIN, PROGESTERONE AND PROSTAGLANDIN E_2 REGULATE INTERLEUKIN 11 DURING HUMAN ENDOMETRIAL STROMAL CELL DECIDUALIZATION

E. Dimitriadis [1], C. Stoikos [1], M. Baca [2], W. D. Fairlie [2], J. E. McCoubrie [2], L. A. Salamonsen [1]

[1] Prince Henry's Institute for Medical Research, Clayton, VIC, Australia
[2] The Walter & Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

Decidualization of endometrial stromal cells is critical for implantation. Interleukin (IL) 11 signaling is essential for embryo implantation in the mouse[1]. The mechanisms of decidualization are poorly understood although molecules including interleukin 11 (IL-11), relaxin (RLX) and prostaglandin (PG) E_2 enhance decidualization. We investigated the secretion of IL-11 during *in vitro* 8-bromo-cyclic adenosine monophosphate (cAMP)-induced decidualization and examined the effect of RLX and PGE_2 on the secretion of IL-11 by non-decidualized human endometrial stromal cells (HESC) for 24 hours (h) and during progesterone (P)- induced decidualization of HESC at 1 and 8 days. cAMP decidualized HESC secreted high levels of IL-11. Addition of either RLX or PGE_2 to HESC increased IL-11 secretion, with a maximal response to RLX treatment at 24h. In contrast, addition of the cAMP/protein kinase (PK) A inhibitor, Rp—adenosine 3,5-cyclic monophosphioate (rp-cAMPs) to either RLX or PGE_2 treated cells, decreased IL-11 secretion although not to control levels. Similarly, addition of indomethacin to the cells decreased IL-11 secretion which was largely restored by co-treatment with PGE_2. Co-treatment of HESC with either RLX or PGE_2 and estrogen (E)+P downregulated IL-11 secretion early in the decidualization process (at 24h) prior to secretion of prolactin (PRL) (a marker of decidualization) but IL-11 secretion was restored by day 8 of decidualization. Addition of W147A, an IL-11 signalling inhibitor, reduced PRL secretion stimulated by either RLX or PGE_2 and E+P. This is the first demonstration that cAMP-decidualized cells secrete IL-11 and that IL-11 secretion from HESC is regulated by RLX and PGE_2, partly via a cAMP/PKA dependent pathway. Furthermore, blocking IL-11 signalling reduced RLX+P or PGE_2+P induced decidualization. The data provides evidence that three key paracrine endometrial factors IL-11, RLX and PGE_2 synergise with P during decidualization. This is important in understanding implantation and regulation of fertility.

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129

EXPRESSION OF PROSTATE SPECIFIC ANTIGEN BY OVARIAN GRANULOSA CELLS.

T. E. Hickey , R. J. Norman

Obstetrics & Gynaecology, Research Centre for Reproductive Health, University of Adelaide, Woodville, SA, Australia

Prostate specific antigen (PSA) is an androgen-regulated serine protease present at elevated levels in the urine of hyperandrogenic women with polycystic ovary syndrome (PCOS), which is a major cause of female infertility[1]. We examined PSA expression in ovarian cells as part of an ongoing investigation into androgen receptor (AR) mediated activity in the human ovary. Two subtypes of granulosa cells were obtained from women undergoing in vitro fertilization procedures: mural granulosa cells (MGCs) purified from follicular aspirates, and cumulus cells (CCs) stripped from the oocyte. Cells were cultured for two days post aspiration in the presence or absence of dihydrotestosterone (DHT), the most potent AR agonist. PSA mRNA expression was determined by RT-PCR and found only in the CCs that had been exposed to androgen; MGCs had no PSA expression in the presence or absence of DHT. Following a 7-14 day culture interval in 10% FCS, CCs proliferated and acquired PSA expression under control conditions (which was further enhanced by addition of DHT) but MGCs did not. Preliminary comparison of a panel of MGCs derived from 16 normal cycling women and 3 women with PCOS reveal PSA expression only in the PCOS group, suggesting an up-regulation of this gene in polycystic ovaries. Dihydrotestosterone could augment insulin-like growth factor-1 (IGF-1) stimulated uptake of tritiated thymidine by CCs but not by MGCs. Here we report for the first time expression of PSA in human ovarian granulosa cells that appears to be restricted to the cumulus cell phenotype under normal circumstances. This evidence suggests that androgens may modulate IGF-1 activity by up-regulating a molecule that is known to cleave IGF-1 binding proteins. Whether PSA is abnormally expressed in PCOS and contributes to the apoptosis resistance characteristic of developmentally arrested follicles within polycystic ovaries requires further investigation.

(1) JCEM (86) 2001

130

EARLY LUTEAL PHASE PROGESTERONE SUPPLEMENTATION AND IVF-ET OUTCOME

C. Haines , L. P. Cheung , P. M. Lam

Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, NT, Hong Kong

The objective of this study was to examine whether the use of early luteal phase progesterone supplementation in addition to standard luteal phase support would improve IVF-ET outcome. It has been suggested that increased uterine contractility may impair endometrial receptivity. Uterine contractility has been found to be negatively correlated with serum progesterone concentrations. Methods: A prospective randomized controlled trial that will include 250 women undergoing IVF treatment. Subjects are randomly assigned to either early (group A) or conventional (group B) luteal

phase support. In group A, 200 mg micronized progesterone is given vaginally three times daily from the afternoon of oocyte retrieval until the morning of embryo transfer (9 doses). Subjects in this group also receive standard luteal phase support in the form of HCG 2000 IU every third day for 4 doses starting on the day of oocyte retrieval. Group B subjects receive HCG luteal phase support as above but no additional progesterone. Results: Biochemical pregnancy rate: 29/68 (42.6%) in Group A and 23/69 (33.3%) in Group B (NS). Clinical pregnancy rate: 24/68 (35.3%) in Group A and 21/69 (30.4%) in Group B (NS). Mean change in serum progesterone 306 ± 117 nmol/L in Group A and 265 ± 131 nmol/L in Group B (NS). Pregnancy outcome: Group A: 19 viable intrauterine, 3 abortion, 2 ectopic, 1 biochemical Group B: 19 viable intrauterine, 2 missed abortion Conclusion: Preliminary data suggest that there is neither an advantage nor a disadvantage in adding vaginal progesterone to standard luteal phase support. Numbers are too few to draw a final conclusion about the difference in pregnancy outcome, but initial results suggest that even if an effect is found, the magnitude of the effect will probably not be clinically significant.

131

GENE EXPRESSION OF OREXIN, MELANIN-CONCENTRATING HORMONE AND NEUROTENSIN ACROSS THE ESTROUS CYCLE OF EWE

K. M. Estrada , A. Rao , S. Pompolo , I. J. Clarke

Neuroendocrinology, Prince Henry's Institute of Medical Res, Clayton, VIC, Australia

A wide variety of neuronal systems have been shown to influence the reproductive axis especially in rodents, but only limited data exist for other species. Furthermore, reproduction is affected by nutritional status and various cell types appear to mediate these effects. Amongst the cells that respond to altered nutrition are those that produce orexin (ORX), melanin-concentrating hormone (MCH) or neurotensin (NT). Each of these peptides exert modulatory effects on gonadotropin releasing hormone (GnRH) and luteinising hormone (LH) secretion in the rat. Our aim was to determine whether gene expression for ORX, MCH and NT alters across the estrous cycle of the ewe. Differing levels of gene expression during the luteal, follicular and estrous phases of the cycle would support a possible role of the relevant gene product as a modulator of GnRH/LH secretion. Brains were collected for *in situ* hybridization from normal cyclic ewes during the luteal and follicular phases of the cycle and at estrus (n=4/group). Using ^{35}S-labelled riboprobes, the number of cells expressing mRNA of each neuropeptide and the number of silver grains/cell was counted. For ORX and MCH, the dorsomedial hypothalamus, perifornical region and the lateral hypothalamic nucleus was examined. NT expression was quantified in the diagonal band of Broca and the preoptic area. There was no significant difference across the estrous cycle in the number of cells expressing ORX, MCH or NT mRNA, nor was there a significant difference in the level of expression/cell. These findings show that, in the ewe, ORX, MCH and NT gene expression does not change throughout the estrous cycle, but this does not exclude the possibility that these neuropeptides regulate GnRH/LH secretion. The data further suggest that expression of these genes is not regulated by sex steroids in the female sheep.

132

A POPULATION BASED STUDY OF THE AGE RELATED DECLINE IN ANDROGEN LEVELS IN AUSTRALIAN MEN

J. Beilin

Diabetes and Endocrinology, Royal Perth Hospital, Perth, WA, Australia

Testosterone levels in males have been reported to decline from the fourth age decade onwards. There is little data on the prevalence of hypogonadism in aging Australian men. This study aims to examine the decline in total and calculated free testosterone in a population based sample of Western Australian men utilising both cross-sectional and longitudinal data with samples obtained from the 1981 Busselton Population Survey (n=910) and 1994/5 follow-up survey (n=483, all of whom were in the 1981 survey). The prevalence of biochemical hypogonadism (reference interval defined by values below the 2.5 centile from young adult men (n=303) <40 years in the 1981 survey) for total testosterone (T) (Immulite 2000, DPC Inc.) (<7.56 nmol/l), calculated non-SHBG T^1 (non-SHBG T)(<4.28 nmol/l) and calculated free T^1 (cFT)(< 0.162 nmol/l) for men greater than 60 years of age is 4.7%, 4.4% and 4.0% in the 1981 survey and 7.0%, 5.0% and 5.2% for the 1994/5 survey. Cross-sectional analysis of both the 1981 and 1994/5 androgen levels show a weak correlation between age and T, cFT and non-SHBG T (r^2 values of 0.02, 0.12 and 0.17 respectively). SHBG levels correlate with age (r^2 0.19) and show a weak inverse correlation with BMI (r^2 0.08) as do T levels (r^2 0.07). The cross-sectional analysis shows cFT and non-SHBG T declines with each age decade (mean decline per decade of 7.7% and 9.0%). Mean androgen levels for men >30 years are lower in 1994/5 compared to 1981(total T 14.0 and 16.2 nmol/L) raising the possibility of an artefact from duration of sample storage, precluding the reliable estimation of longitudinal rate of decline of androgen levels. This Australian population based study reports a slower rate of decline in total and bioavailable testosterone and lower prevalence of biochemical hypogonadism than previously reported for other populations.

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133

DEVELOPMENT AND VALIDATION OF MODIFIED SILASTIC IMPLANTS TO DELIVER PHYSIOLOGICAL BLOOD ESTRADIOL (E2) CONCENTRATIONS IN MICE.

J. Spaliviero , M. Jimenez , T. Borovina , C. Allan , D. Handelsman

Andrology laboratory, ANZAC Research Institute, Sydney, NSW, Australia

Silastic subdermal implants constitute a convenient and effective depot delivery system for reproductive steroids in rodents to maintain steady-state blood steroid levels proportional to implant length for weeks to months. However, implants filled with E_2 produce markedly supraphysiological blood E_2 concentrations and weight loss that is not satisfactory to study pharmacological or physiological estrogen effects. We therefore developed modified Silastic implants to deliver more physiological E_2 blood levels using cholesterol-diluted E_2 to deliver lower daily doses and maintain physiological blood E_2 concentrations. Silastic implants (1 cm, OD 1.95mm, ID 1.47mm) filled with E_2 recrystallized from ethanol in a range of dilutions with cholesterol (1/3000, 1/1000,1/300, 1/100,

1/30 & 1/10) were implanted for 2 weeks into mature male mice (n=5-6/group). Serum E_2 (101 ± 7, 83 ± 4, 204 ± 18, 429 ± 29, 525 ± 22, 1433 ± 44 pM respectively) showed a dose response relationship with E_2 dose in intact male mice. None of the E_2 doses had any effect on body weight. Castrate male mice showed a consistent dose response relationships for up to 9 weeks of E_2 treatment (1/1000 dilution) when implanted with 1 implant (3W: 290 ± 16pM, 6W: 233 ± 11pM, 9W: 215 ± 7pM) or 2 implants (3W: 403 ± 57pM, 6W: 440 ± 61pM, 9W: 321 ± 20pM) compared with unimplanted castrate mice (173 ± 10 pM) and maintaining physiological levels comparable with intact mice (193 ± 6 pM). We conclude that this modified Silastic implant estradiol delivery system provides a convenient and practical method to sustain physiological blood E_2 concentrations for 6-9 weeks in male mice.

134

TESTOSTERONE LEVELS IN MEN WITH CHRONIC HEPATITIS C INFECTION: EFFECT OF DISEASE SEVERITY AND RECOMBINANT INTERFERON-ALPHA THERAPY.

H. Nguyen [1], T. Taylor [3], L. C. Mollison [4], P. Chubb [3], B. B. Yeap [1,2]

[1] Endocrinology, Fremantle Hospital, Perth, WA, Australia

[2] School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia

[3] Biochemistry, Fremantle Hospital, Perth, WA, Australia

[4] Gastroenterology, Fremantle Hospital, Perth, WA, Australia

BACKGROUND/AIMS : Study aims were to assess the prevalence of reduced serum testosterone in men with chronic hepatitis C, explore associations between androgen status and markers of liver disease severity and determine the effect of interferon alpha (IFN-α) treatment on hormone levels. METHODS : We audited a clinical database of 77 men with chronic hepatitis C attending Fremantle Hospital over twelve months. RESULTS : 5.2% had serum total testosterone <8 nmol/L. Free testosterone levels inversely correlated with liver Fibrosis Score measured by Knodell histological activity index (HAI) (r = -0.45, *P*=0.005). Sex Hormone Binding Globulin directly correlated with Fibrosis Score (r = 0.59, *P*=0.0001) and total HAI score (r = 0.35, *P*=0.037). A transient reduction in total testosterone of 5.7 nmol/L occurred within the first six months of IFN-α therapy in 11 patients, with free testosterone unaffected. Total testosterone before, during and after IFN-α were 24.4±8.4 , 18.7±3.5 and 22.0 ±4.5 nmol/L (*P*<0.05). CONCLUSIONS : More severe disease measured by Fibrosis Score is associated with lower serum free testosterone. IFN-α therapy reduces serum total testosterone but not to androgen deficient levels. These findings support selective screening of men with more severe hepatitis C infection for symptoms and biochemical evidence of hypogonadism.

136

GENERATION OF SKELETAL MUSCLE-SPECIFIC ANDROGEN RECEPTOR KNOCKOUT MICE

H. E. MacLean , A. J. Notini , A. Axell , R. A. Davey , J. F. McManus , S. J. Kaczmarczyk , J. Favaloro , J. Proietto , J. D. Zajac

Medicine (Austin Hospital), University of Melbourne, Heidelberg, VIC, Australia

We are studying the mechanisms via which androgens increase muscle bulk and strength. We previously showed that castration of male mice reduces muscle mass and force output, and these effects are prevented by testosterone supplementation. To investigate the target cells of androgen action, we are using cre/lox to generate skeletal muscle-specific androgen receptor (AR) knockout mice. We have generated a floxed AR mouse line, in which exon 3 of the AR gene is flanked by lox sites. We are using two muscle-specific cre mouse lines. We have created the α-actin-cre line, in which the cre transgene is expressed in proliferating myoblasts and mature myofibres. We also have the muscle creatine kinase-cre line, in which the cre transgene is expressed only in mature myofibres. To assess cre expression in the α-actin-cre line, rosa reporter mice were mated with the α-actin-cre mice, to generate offspring in which *lacZ* is expressed in tissues only where the cre enzyme is active. Newborn pups stained for β-galactosidase activity showed all skeletal muscles were blue, indicating the α-actin-cre transgene is functional in all muscles. We are currently back-crossing all lines onto a C57BL6 background. We have generated muscle-specific AR knockout mice of mixed genetic background, to assess the tissue-specificity of cre-mediated deletion. The deleted AR allele is present in DNA from skeletal muscles of 7 w.o. mice, and is not deleted in DNA from the kidney. The AR is detectable by Western analysis in skeletal muscle, because the exon 3 deletion maintains the reading frame of the AR gene. When pure C57BL6 muscle-specific AR knockouts are obtained, their muscles will be characterised by examining muscle mass, fibre size and muscle contractile properties. These studies will identify the effects of androgens acting through the AR in myoblasts and myofibres, the predominant cell types in skeletal muscle.

137

GENERATION OF AN ANDROGEN RECEPTOR KNOCKOUT MOUSE LINE USING THE CRE/LOXP SYSTEM

A. J. Notini , R. A. Davey , J. F. McManus , K. L. Bate , J. D. Zajac

Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, VIC, Australia

Androgens mediate their effects in target cells via an interaction with the androgen receptor (AR), which directly modulates target gene expression. We have generated a mouse model using the Cre/loxP system to disrupt AR function in a tissue-specific manner. A region of the mouse AR gene containing exon 3 was cloned and characterised from a 129SV/J mouse genomic library and used to generate a targeting construct, in which, exon 3 was flanked by loxP sites. Exon 3 encodes the 2nd zinc finger of the DNA binding domain of the AR, which plays a critical role in stabilising receptor-DNA interaction and receptor dimerisation. The AR-loxP targeting construct was electroporated into 129SV/J mouse embryonic stem (ES) cells and 200 neomycin resistant ES cell colonies were selected and screened for homologous recombination events by PCR. 16 positive ES cell clones were identified and microinjected into C57Bl6 mouse blastocysts. Male chimeras were backcrossed to female C57Bl6 mice and offspring genotyped by PCR. All agouti female offspring demonstrated

inheritance of the floxed allele thus verifying germline transmission. In order to confirm that deletion of exon 3 renders the AR non-functional, AR floxed mice were crossed with CMV-Cre mice, which express Cre recombinase ubiquitously. AR knockout XY males displayed a complete androgen insensitivity phenotype, with female external genitalia and body weight. The testes in AR knockout males were 95% smaller than wildtype or floxed littermate controls ($p<0.001$) and were located abdominally. Unlike other AR knockout models, Cre-mediated deletion of the floxed allele in our mice does not result in a frameshift mutation. Based on the studies of a pair of human siblings with complete androgen insensitivity (cAIS) due to deletion of exon 3, we expect that the AR in our knockout mice is able to bind androgens but not genomic DNA and therefore unable to regulate transcription of target genes.

138

REGULATION OF THE EXPRESSION OF OREXIN RECEPTORS (1 AND 2) AND LEPTIN RECEPTORS (A AND B) BY OESTROGEN IN THE AROMATASE KNOCK-OUT (ARKO) MOUSE

M. Yan , M. E.E. Jones , G. Nie , D. Liu , E. R. Simpson , C. Chen

PHIMR, Prince Henry's Inst of Medical Research, Melbourne, VIC, Australia

The effects of leptin and orexins on growth hormone (GH) secretion have been reported both in vitro and in vivo in different species and under different nutritional conditions. Orexins stimulated, whereas leptin inhibited GH secretion from primary cultured ovine somatotropes through their specific receptors on the cell membrane (1, 2). The regulation of GH levels by estrogen (E2) have been studied but levels of orexin receptors and leptin receptors in the pituitary gland in an E2-deficient condition are unknown (3). In the present study, we investigated the influence of E2 on the expression of orexin receptors 1 and 2, and leptin receptors A and B in the pituitary gland. Orexin receptor and leptin receptor mRNA levels were measured using semi-quantitative RT-PCR with GAPDH as an internal control. Aromatase knock-out (ArKO) mice lack aromatase and are therefore oestrogen deficient. The levels of pituitary orexin receptor 1 mRNA were elevated in adult ArKO mice compared with wild type (WT) controls. E2 replacement for 21 days decreased pituitary orexin receptor 1 mRNA in adult ArKO mice. The levels of orexin receptor 2 mRNA were not significantly changed in adult ArKO mice compared with appropriate WT controls. The levels of pituitary leptin receptor A and B mRNA did not change in adult ArKO mice. However, E2 replacement for 21 days reduced pituitary leptin receptor A, but not leptin receptor B, mRNA levels in adult ArKO mice. These results indicate that E2 replacement suppresses pituitary orexin receptor 1 and leptin receptor A mRNA expression in adult ArKO mice. Functional implications warrant further investigation. Supported by NHMRC.

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FUNCTIONAL CHARACTERISATION OF OREXIN RECEPTOR SUBTYPES 1 AND 2 USING BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET) AND CONFOCAL MICROSCOPY

M. B. Dalrymple [1,2,3], K. D.G. Pfleger [1], K. A. Eidne [1]

[1] WAIMR/UWA Centre for Medical Research, University of Western Australia, Perth, WA, Australia

[2] School of Anatomy, University of Western Australia, Perth, WA, Australia

[3] Keogh Institute for Medical Research, QE2 Medical Centre, Perth, WA, Australia

The role of orexin neuropeptides and their cognate G-protein coupled receptors (GPCRs) was initially believed to be involved in mediation of feeding behaviour, providing an intriguing candidate for the treatment of eating disorders. However it has recently become apparent that the role of the orexin system may be considerably more diverse and complex, with several lines of evidence indicating its involvement in regulating vigilance and the sleep/wake cycle. Particularly interesting is the possibility that the orexin system may play a significant part in the pathogenesis of the sleep condition narcolepsy. Using bioluminescence resonance energy transfer (BRET) in live cells, in real time, we are able to evaluate dimerization between orexin receptors, as well as interactions with the trafficking proteins beta-arrestin 1 and 2. Results show that both human orexin receptor 1 (hOXR1) and 2 (hOXR2) are capable of forming homodimers, and in addition, are able to form heterodimers between receptor subtypes. In both cases these interactions are constitutive, with addition of ligand appearing to have little effect. With regard to receptor trafficking and desensitization, we are also able to demonstrate that internalization of hOXR1 and hOXR2 subtypes is promoted by both beta-arrestin 1 and 2 in a ligand-dependent manner. Moreover, these receptors interacted directly with each arrestin to a similar extent, suggesting that both belong to the Class B family of GPCRs. The role of receptor phosphorylation was investigated by examining the ability of the OXRs to directly interact with phosphorylation-independent arrestins. Arrestin-dependent desensitization of orexin receptors was substantiated with confocal microscopy, using GFP-tagged arrestin or receptor constructs. Given that there is existing evidence of orexin receptors co-localising in the paraventricular and ventromedial nuclei of the rat hypothalamus, our results showing orexin receptor heterodimerization may help provide a better understanding of their homeostatic role.

140

A COMPARATIVE STUDY OF RENILLA LUCIFERASE SUBSTRATES AND THEIR APPLICATION TO REAL-TIME BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET)

K. D.G. Pfleger [1], E. Lim [1], M. B. Dalrymple [1,2,3], U. Schmidt [1], S. M. Szefczyk [1], K. A. Eidne [1]

[1] WAIMR/UWA Centre for Medical Research, University of Western Australia, Perth, WA, Australia

[2] School of Anatomy, University of Western Australia, Perth, WA, Australia

[3] Keogh Institute for Medical Research, QE2 Medical Centre, Perth, WA, Australia

Bioluminescence resonance energy transfer (BRET) is a system that enables protein-protein interactions to be studied in live cells, in real-time. Proteins of interest are fused to either an energy donor (Renilla luciferase, Rluc) or acceptor (a variant of green fluorescent protein, GFP). Following transient or stable expression of these fusion proteins in cell lines, oxidation of substrate by Rluc results in energy emission, which is transferred to the GFP variant if the donor and acceptor are in close proximity (within 100 A). Therefore, consequent energy emission from the GFP variant at a distinct wavelength (510-590nm) is indicative of an interaction between the proteins of interest, and is usually presented as a ratio of the energy resulting from Rluc activity (wavelength of 440-500nm for coelentrazine h). This study uses both scanning spectrophotometry and BRET to compare various Renilla luciferase substrates, including coelentrazine h and EnduRen™, in terms of spectra, intensity and stability. Particular emphasis is placed on establishing their suitability for real-time BRET kinetics. EnduRen™ is a protected form of the cell-permeable coelentrazine h that is metabolised to the free substrate within the cell. Consequently, extracellular substrate degradation and autoluminescence is substantially reduced. Using interactions involving G-protein coupled receptors, we demonstrate that the stability of EnduRen™ enables real-time BRET kinetic studies to be carried out over several hours, in contrast to the short time scales achieved with unprotected substrate. This represents a significant and exciting advance in the BRET technology, adding another dimension to our ability to investigate dynamic protein-protein interactions in a near-physiological environment.

141

THE METABOLIC SYNDROME IN ASIA: PREVALENCE AND RISK FACTORS

A. Omari [1], K. Huang [2], N. Kormas [1], I. D. Caterson [1,3]

[1] Dept. of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

[2] Dept. of Family Medicine, National Taiwan University Hospital, Taipei, Taiwan

[3] Human Nutrition Unit, University of Sydney, Sydney, NSW, Australia

Objectives: To determine the prevalence of the metabolic syndrome and its individual components in a large Asian population. *Methods:* A cross-sectional study of 72,223 adults aged 20 years and older from a nationwide Taiwanese Health Centre survey (1999) was conducted. The National Cholesterol Education Program (NCEP) criteria were used to define the metabolic syndrome(1). *Results:* The metabolic syndrome was present in 7% of the study population. The prevalence of the metabolic syndrome increased with age, males having a higher prevalence under 50 years of age and women having a higher prevalence over 50 years of age. Amongst Taiwanese adults, 55.5% of men and

46.4% of women did not have any criteria of the metabolic syndrome. A marked variation in the prevalence of individual criteria was present in the Taiwanese population. Increased triglycerides were found in 23.8% of the study population and decreased HDL cholesterol in 35.2%. High fasting glucose and elevated blood pressure were present in 9.7% and 9.9% respectively. Abdominal adiposity was found in only 3.3% of the population. *Conclusions:* The metabolic syndrome is a common condition amongst Taiwanese adults, increasing with age in each gender. A significant proportion of Taiwanese adults did not exhibit any features of the metabolic syndrome. However, the uneven distribution of individual components such as the disproportionately high prevalence of dyslipidaemia and low prevalence of central obesity indicate that current definitions may not adequately define the metabolic syndrome in Asian adults and possibly explains why the prevalence of the metabolic syndrome is much lower than in Caucasian adults in developed countries. This study suggests that ethnic specific criteria will need to be developed to define the metabolic syndrome in Asian populations.

(1) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive

142

CHANGES IN ANDROGEN LEVELS ACROSS THE ADULT FEMALE LIFE SPAN AND RELATIONSHIPS TO SEXUAL FUNCTION

S. L. Davison [1], R. J. Bell [2], S. Donath [2], J. G. Montalto [3], S. R. Davis [2]

[1] Biochemistry, Monash University, The Jean Hailes Foundation Research Unit, Clayton, VIC, Australia

[2] Obstetrics and Gynaecology, Monash Uni, The Jean Hailes Foundation, Clayton, VIC, Australia

[3] Biochemistry, Mayne Health Dorevitch Pathology, Heidelberg, VIC, Australia

Background and aims: The aims of this study were to determine ranges by decade for total testosterone (T), calculated free T^1, DHEAS, and androstenedione (A) in healthy women, to explore the effects of gynaecological surgery and menopause on androgens, and to evaluate the relationships between androgens and sexual function. Methods: 1423 Victorian women, aged 18 to 75 years, were randomly recruited from the community. Each provided a morning blood sample and completed validated health and sexual function questionnaires. Premenopausal women had blood drawn after cycle day 8. Hormones were measured using sensitive assays. A reference population was created, which excluded women with use of exogenous steroids, bilateral oophorectomy, hysterectomy, tubal ligation, hyperprolactinemia, or polycystic ovarian syndrome to establish androgen values by decade. Results: 595 women fulfilled the criteria for the reference group. In this population all androgens declined steeply by decade ($P<0001$), with the decline being greatest in the younger decades. An examination of androgen levels by year in women aged 45-54 years revealed no obvious difference between women who were premenopausal vs. postmenopausal. Bilaterally oophorectomised women >55 years, not on exogenous steroids, had significantly lower T levels than reference women of the same age. Of the androgens measured, only DHEAS levels were significantly associated with low sexual desire, arousal and responsiveness in women < 45 years. Conclusions: We report normative data for androgens in women and demonstrate that DHEAS, androstenedione, total and free T levels decline steeply in the early reproductive years, and do not vary with natural menopause. Bilateral oophorectomy results in lower T levels in postmenopausal women, indicating ongoing ovarian T production beyond the menopause. We also report that DHEAS levels make an independent contribution to low levels of sexual interest, arousal and responsiveness in younger women.

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143

VASCULAR DYSFUNCTION AND METABOLIC PARAMETERS IN PCOS

C. Meyer , B. P. McGrath , D. Kotsopoulos , H. J. Teede

Vascular Medicine, Dandenong Hospital, Dandenong, VIC, Australia

Aims: To assess the relationships between arterial structure and function, androgen status, insulin resistance and other metabolic parameters in PCOS. Methods: Arterial structure and function was assessed in 80 overweight women with PCOS including structural measures (carotid intimal media thickness [IMT]) and functional measures (pulse wave velocity [PWV] and flow mediated vasodilation [FMD]). Metabolic parameters included insulin and glucose during an OGTT, lipid parameters and serum androgens. Results: Multiple regression analysis showed that after adjusting for age, DHEA was negatively correlated with IMT (p=0.01) whereas BP load was positively correlated (p=0.02). Higher tertiles of androgens corresponded to significantly lower IMT. Multiple regression analysis showed that FMD was positively related FAI (p=0.008) and negatively related to lipid parameters (p=0.005) whilst PWV was positively related to blood pressure (p<0.001), AUC insulin (p=0.04) and AUC BGL (p=0.035) after adjustment for age. Conclusion: In overweight women with PCOS insulin resistance and blood pressure have a deleterious effect on arterial stiffness. As a novel hypothesis we propose that serum androgens may afford some protection from the increased CV disease burden that might be expected in this group of otherwise high CV risk women. Further research is needed to clarify and support these findings, including studying the arterial structural and functional effects of pharmacological modulation of androgen and insulin status in women with PCOS.

144

MANAGEMENT OF SUBCLINICAL HYPOTHYROIDISM - AN AUDIT

M. J. Gillett [1], C. Yeo [1,2], S. D. Vasikaran [1]

[1] Core Clinical Pathology and Biochemistry, Royal Perth Hospital, Perth, WA, Australia

[2] Department of Pathology, Singapore General Hospital, Singapore, Singapore

Introduction: The aim of this study was to audit the management of subclinical hypothyroidism in a hospital setting. Methods: A retrospective audit of clinical case notes was conducted in December 2003 of patients with TSH between 4.1 – 9.9 mIU/L and FT4 within the reference interval in November 2002. The patients' general practitioners were contacted for additional information. Results: There were 72 patients with subclinical hypothyroid results in November 2002. We excluded 29 patients from other hospitals and 3 whose general practitioners were not contactable. Of the remaining 43 patients, 18 [8 males and 10 females, age range 24 to 90] had no previous history of thyroid disease. All the laboratory reports of subclinical hypothyroid results were accompanied by a comment that advised repeat thyroid studies and thyroid antibody tests at a later date. However, only 3 of the 18 patients had thyroid peroxidase antibodies (TPOAb) checked. Only 7 of the 18 patients were followed up with repeat thyroid function tests, at intervals ranging from 3 days to 7 months. One of these patients with a TSH of 9.8 was started on replacement therapy. General practitioners of 10 out of the 11 patients who did not have follow up testing were not informed of the initial TSH results. Discussion: Only 7 out of 18 patients with a new diagnosis of subclinical hypothyroidism had follow-up thyroid function tests in the next 12 months, and only 3 had TPOAbs tested. The low rate of follow up is of concern. Better strategies to improve follow-up of patients who return subclinical hypothyroid results in a hospital setting may be warranted. Laboratory generated reflex testing of TPOAbs and inclusion of treatment advice dependent on TPOAb status in reports are possible measures.

145

THYROID DYSFUNCTION AS A PREDICTOR OF CARDIOVASCULAR DISEASE AND MORTALITY: AN ANALYSIS FROM THE BUSSELTON HEALTH STUDY.

J. P. Walsh [1], P. O'Leary [2], P. J. Leedman [3,4,5], V. Michelangeli [6], P. Feddema [6]

[1] Dept of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
[2] Genomics Directorate, Department of Health, WA, Australia
[3] WA Institute for Medical Research, Perth, WA, Australia
[4] School of Medicine and Pharmacology, University of Western Australia, WA, Australia
[5] Dept of Endocrinology & Diabetes, Royal Perth Hospital, WA, Australia
[6] BioMediq DPC, Melbourne, VIC, Australia

Thyroid hormones have profound effects on the cardiovascular system, but there are few large epidemiological studies examining the effects of thyroid dysfunction on cardiovascular disease. In particular, there are conflicting data as to whether subclinical hypothyroidism increases cardiovascular risk. We examined the association between thyroid dysfunction, cardiovascular disease and mortality rates in a community-based study in Western Australia. Serum TSH (reference range 0.4-4.0 mU/L) and free T4 concentrations were measured on 2115 samples stored from the 1981 Busselton Health Survey. In a cross-sectional study, thyroid function was correlated with cardiovascular risk factors and the presence of coronary artery disease in participants. In a longitudinal study, vital status and hospital admission up to the end of 2001 have been determined from the WA Hospital Morbidity Data System. In females, serum TSH showed a strong positive correlation with total cholesterol ($P<0.0001$) and triglycerides ($P<0.0001$). In males, TSH was correlated with triglycerides ($P=0.025$), but not with cholesterol. There was no significant correlation between TSH and HDL cholesterol. Total cholesterol was significantly higher in subjects TSH>4mU/L compared to euthyroid subjects (mean ± SD 6.34 ± 1.37 vs. 5.80 ± 1.17, $P<0.001$), as was serum triglycerides (1.65 ± 1.31 vs. 1.44 ± 1.03, $P=0.032$). In subjects with subclinical hypothyroidism, serum cholesterol was significantly higher than euthyroid subjects (6.27 ± 1.28 vs. 5.80 ± 1.17, $P<0.001$), as was triglycerides (1.68 ± 1.38 vs 1.44 ± 1.03, $P<0.01$). We conclude that subclinical hypothyroidism is associated with significant elevations in serum total cholesterol and triglycerides. The effects of subclinical hypothyroidism on cardiovascular disease and overall mortality in this population remain to be determined.

146

AMIODARONE-INDUCED THYROTOXICOSIS: FACTORS WHICH EFFECT RECOVERY AND OUTCOME

A. J. O'Sullivan [1], M. Lewis [2], T. Diamond [2]

[1] Medicine, University of New South Wales, Kogarah, NSW, Australia
[2] Endocrinology, St. George Hospital, Sydney, NSW, Australia

Amiodarone-induced thyrotoxicosis (AIT) is a challenging management problem, since patients treated with amiodarone invariably have underlying heart disease. Consequently, thyrotoxicosis can significantly contribute to increased morbidity and mortality. The aim of the study was to compare AIT (n=54) to Graves thyrotoxicosis (n=49) and toxic multinodular goitre (MNG, n=37) and to determine which factors in AIT effects recovery (time to normalise free T4) and outcome. A

retrospective study of patients with AIT in a single institution was conducted. Patients with Graves disease were significantly younger (Graves disease vs toxic MNG vs AIT, 44 ± 2 # vs 74 ± 2 vs 68 ± 2 years, $p<0.01$) and free T3 levels were higher (13.6 ± 1.6 # vs 7.3 ± 0.6 vs 9.2 ± 1.1, # $p<0.01$). TSH and free T4 levels were not different between groups. Free T4/free T3 ratio was significantly higher in AIT consistent with reduced conversion of T4 to T3 (3.38 ± 0.21 vs 3.40 ± 0.2 vs 6.20 ± 0.49, $p<0.01$). Recovery was significantly delayed in AIT (13 ± 2 vs 9 ± 1 vs 28 ± 4 weeks, $p<0.01$). AIT was a significant predictor of death ($p=0.002$) whereas free T4 levels and age were not, as 6 patients with AIT died before recovery whereas no other patients died. In the AIT group, 12 patents had severe left ventricular dysfunction including the 6 patients who died ($p<0.01$). Within the AIT group, free T4 levels were not related to duration of amiodarone therapy or total amiodarone dose or time to recovery or left ventricular dysfunction. We conclude that severe left ventricular dysfunction is an important prognostic factor for AIT whereas age, degree of thyrotoxicosis and amiodarone dose are not.

147

GENOTYPE-PHENOTYPE ANALYSIS AND EXTENSIVE EXPERIENCE OF PROPHYLACTIC THYROIDECTOMY IN A VERY LARGE MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A KINDRED WITH THE RET CODON 804 MUTATION.

D. L. Learoyd [1], J. Gosnell [2], A. Richardson [4], L. Lim [4], T. Saurine [3], J. Philips [3], L. W. Delbridge [2], B. G. Robinson [1]

[1] Medicine (Endocrinology), RNSH and University of Sydney, St Leonards, NSW, Australia
[2] Surgery, Royal North Shore Hospital, St Leonards, NSW, Australia
[3] Anatomical Pathology, Royal North Shore Hospital, St Leonards, NSW, Australia
[4] Cancer Genetics Laboratory, Kolling Institute RNSH and Uni of Sydney, St Leonards, NSW, Australia

Genetic screening in patients with multiple endocrine neoplasia type 2 (MEN 2) has been widely available for the last decade and all medullary thyroid carcinoma (MTC) cases are screened for *RET* mutations, but only recently has genetic analysis been offered to all phaeochromocytoma cases. Comprehensive genetic screening leads to identification of *RET* mutations in 99% of true MEN 2 families. MTC spreads early to lymph nodes and thus it is recommended that asymptomatic *RET* mutation carriers should undergo prophylactic thyroidectomy from the age of 5 years, but this is difficult for families to accept. Some authors have reported a less aggressive phenotype for the exon 14 V804L *RET* mutation suggesting that prophylactic surgery could be delayed until adulthood, but this is controversial. We report an extensive genetic and clinical analysis of one of the largest MEN 2 families in the literature, bearing the V804L *RET* mutation. The proband's unusual presentation at age 59 with a large ruptured phaeochromocytoma led to a clinical diagnosis of MTC and subsequent confirmation of the codon 804 *RET* mutation. Genetic screening has been performed on more than 46 members of this family uncovering 23 mutation carriers thus far in 4 generations. Biochemical screening for baseline serum calcitonin, calcium, parathyroid hormone (PTH) and urinary catecholamines was performed in all gene carriers and prophylactic thyroidectomy has been performed in 18 asymptomatic gene carriers thus far. A routine central lymph node clearance is performed at thyroidectomy and final pathology has revealed MTC in all gene carriers of the proband's generation (ages 59 to 62) except two who had C cell hyperplasia (CCH). In younger generations (ages 10 to 43) 11 thyroidectomies thus far have revealed 4 cases of MTC and 6 cases of

CCH. Lymph node metastases and phaeochromocytoma have only been found in the proband although several cases of hyperparathyroidism have been uncovered. There are important lessons from analysis of this family. The clinical presentation of this mutation is variable with phaeochromocytoma being uncommon and MTC occurring as early as 12 years. Other issues discussed are the prevalence of papillary microcarcinoma, and of the codon 836 *RET* polymorphism, and the huge effort involved in tracking down family members and persuading them to have thyroidectomy!

148

MATERNAL SERUM FOLLISTATIN CONCENTRATIONS ARE INFLUENCED BY FOETAL SEX.

K. M. Rae , K. G. Hollebone , M. S. Baylis , D. C. Clausen , V. Chetty , J. R. McFarlane

Biological, Biomedical and Molecular, University of New England, Armidale, NSW, Australia

Current evidence suggests that follistatin is likely to have a role in pregnancy, and parturition. In this study we examined the effect of fetal sex on maternal plasma concentrations of follistatin across late pregnancy, labour and the postpartum period. Women who underwent vaginal delivery after a normal pregnancy (n=36) were retrospectively divided into 2 groups determined by fetal sex; male babies (n=15) and female babies (n=21). The mean gestation and fetal weights were comparable (39.1 weeks to 39.6 weeks and 3343g to 3244g for male to female groups respectively). Blood samples were taken at late gestation, once during labour, and in the 24 hour postpartum period. A cord blood sample was taken at birth. Follistatin was measured as previously described (1). Follistatin showed significant differences in the labouring samples between the sexes. Mothers carrying male offspring had significantly higher serum follistatin concentrations when compared with those carrying females (42.3ng/ml + 14.8 versus 22.65ng/ml + 3.61). However the sex related differences were only noted in the labouring period. In the antenatal and postpartum samples no significant differences was seen between groups. Interestingly, cord blood samples showed slight differences between sex and at concentrations significantly lower than those in maternal circulation. These novel results show there is a rise in follistatin related to fetal sex during labour. The low levels of follistatin in fetal samples suggest that the values observed in maternal serum are due to a maternal expression of follistatin in response to the fetal sex. Further studies are underway to test this hypothesis.

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149

FAMILIAL PAPILLARY THYROID CARCINOMA

W. E. Plehwe

The Epworth Centre, Richmond, VIC, Australia

A family is reported in which thyroid abnormalities alone occurred in 5 generations including Papillary Carcinoma (PTC) in 3 members; a mother, her daughter and son. The daughter (index case) presented aged 36y with a thyrotoxic multinodular goitre (MNG) and was controlled medically before proceeding to surgery. Histology revealed a 2.0mm nodule of PTC fully excised. She received suppressive T4 and has remained well for 10y.

In 1962 her mother required extensive surgery followed by I-131 for thyroid carcinoma and has remained disease-free. All her 4 maternal aunts and 1 of 5 maternal uncles had MNG. Her brother presented in 2002 with a thyroid nodule (follicular adenoma) but at surgery also had PTC metastatic to ipsilateral cervical lymph glands. He underwent total thyroidectomy and lymph gland clearance followed by ablative I-131. T4-suppressed pre- and post-ablation thyroglobulin were 7 and <1.4 pmol/L. Follow-up total body I-131 scan will follow in July 2004.

Her maternal grandmother and great-grandmother had large MNG. Her sons (18, 20y) presented in late 2003 with mildly elevated TFT. Each had thyroid nodules (1.0cm, and 2.0 and 3.0mm respectively) present ultrasonographically but TFTs have normalised and clinical and ultrasound monitoring continues. Her father has received I-131 for a toxic thyroid nodule. The family have never resided in an iodine-deficiency area. Genetic analyses will be undertaken.

Since the initial report of familial PTC (1), associations between PTC and uncommon syndromes (Familial Adenomatous Polyposis, Gardner's and Cowden's syndromes) have been noted. Prevalence studies suggest that up to 6% of PTC patients may have at least 1 first degree relative with PTC and that familial PTC may be more aggressive than sporadic cases. Since standard surgical and I-131 therapy are successful in eradicating the disease, monitoring of first degree relatives of PTC cases by annual neck palpation and ultrasound should be considered.

(1) Nemec J et al. Oncology (1975); 32:151-7.

150

INVESTIGATIONS INTO THE DOMAINS OF THE RELAXIN-1 (LGR7) AND INSL3 (LGR8) RECEPTORS RESPONSIBLE FOR LIGAND BINDING AND ACTIVATION.

R. A.D. Bathgate [1], S. Sudo [2], S. Layfield [1], D. Scott [1], E. Hopkins [1], T. Ferraro [1], G. Tregear [1], A. J.W. Hsueh [2]

[1] Howard Florey Institute, University of Melbourne, VIC, Australia

[2] Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California, United States

The Leucine-rich Repeat-containing G-protein-coupled-receptor (LGR) family includes the glycoprotein hormone receptors (LHR, FSHR, TSHR) and are characterized by a large extracellular ectodomain. Recently, the newest family members, LGR7 and LGR8, were demonstrated to be the receptors for relaxin-1 and Insulin-like peptide-3 (INSL3) respectively. Relaxin-1 is essential for pregnancy while INSL3 is essential for testis descent and germ cell maturation. LGR7/8 form a LGR sub-family and contain a unique N-terminal Low-Density-Lipoprotein Class-A (LDLa) domain. Although data from *in vivo* studies and knockouts indicate that the two receptors have distinct

functions there is cross-reactivity of relaxin peptides on both receptors. As relaxin peptides are being developed for clinical applications it is essential to understand their interactions with these receptors. We have utilized chimeras of LGR7 and LGR8, single transmembrane anchored ectodomain only receptors (7BP & 8BP) as well as native splice variants of LGR7 and LGR8 to study the determinants of ligand binding and activation. LGR7/8 mutants and native splice variants missing the LDLa domain bind ligand but do not activate cAMP signaling. Hence the LDLa domain is essential for receptor signaling and the leucine-rich repeats (LRRs) are the sites of primary ligand binding. LGR7/8 chimeras with swapped ectodomains indicate that there is a secondary binding site in the transmembrane (TM) exoloops which is necessary for signaling. Membrane anchored receptors 7BP and 8BP, missing these exoloops, demonstrate high affinity binding indicating this site is not essential for primary binding. We therefore have a basic model of LGR7/8 function that involves ligand binding to a primary site in the LRRs followed by a secondary binding site in the TM-exoloops. The LDLa domain is then essential for ligand directed activation. These studies are essential for the design of the next generation of relaxin peptide agonists and antagonists for applications in modulating reproductive function.

151

ANTAGONISM OF ACTIVIN TYPE II RECEPTORS

C. A. Harrison [1,2], **P. C. Gray** [2], **W. H. Fischer** [2], **W. W. Vale** [2]

[1] ***Reproductive Hormones Group, PHIMR, Clayton, VIC, Australia***

[2] ***Peptide Biology Laboratories, The Salk Institute, La Jolla, California, United States***

Activins control many physiologic and pathophysiologic processes in multiple tissues and, like other TGFβ superfamily members, signal via type II (ActRII/IIB) and type I (ALK4) receptors. ActRII/IIB are promiscuous receptors that bind 12 TGFβ ligands including activins, myostatin and BMPs. In this study, we generated a screen for identifying activin mutants with loss of signaling activity. Our goal was to identify activin variants able to bind ActRII but unable to bind ALK4 and therefore candidate type II activin receptor antagonists. Using the structure of BMP2 bound to its type I receptor (ALK3) as a guide, we introduced mutations in the context of the inhibin βA construct and assessed the signaling activity of the resulting activin mutants. We identified several mutants in the finger (M91E, I105E, M108A) and wrist (activin A/activin C chimera, S60P, I63P) regions of activin-A with reduced signaling activity and showed that four of these mutants retained high affinity binding to ActRII. Of these the activin-A (M108A) mutant displayed the lowest signaling activity while retaining wild type-like affinity for ActRII. Unlike wild type activin, the M108A mutant was unable to form a crosslinked complex with ALK4 in the presence of ActRII indicating that its ability to bind ALK4 was disrupted. This data suggested that the M108A variant might be capable of modulating signaling of activin and related ligands. Indeed, the M108A mutant blocked activin-A and myostatin, but not TGFβ , signaling in 293T cells, indicating it may be generally capable of antagonizing ligands that signal via type II activin receptors. In a more physiological system, the M108A mutant also blocked FSH release by the LβT2 gonadotrope cell line. Several human disorders have the potential to be treated by type II activin receptor modulators and/or antagonists including: (1) muscular dystrophy, (2) cachexia, (3) wound healing, (4) liver regeneration and (5) cancer.

152

ESTROGEN ACTIONS AND SIGNALLING MECHANISMS IN CULTURED RAT PELVIC AUTONOMIC AND SENSORY NEURONS.

T. D. Purves-Tyson , J. R. Keast

Prince of Wales Medical Research Inst, University of New South Wales, Randwick, NSW, Australia

Sensory and pelvic autonomic ganglia innervating the urogenital tract express estrogen receptors (ER) and are potentially modulated by circulating steroids. Signalling mechanisms activated by estradiol and physiological effects of estradiol (E2) have not been identified in the peripheral nervous system. Activation of the transcription factor cyclic AMP response element binding protein (CREB) by the extracellular signal-regulated kinase (ERK) and/or phosphatidylinositol-3 kinase (PI3K) pathways is a key mechanism mediating the effects of neurotrophins. Our aims were to elucidate the signalling pathways activated by steroids and identify the physiological outcomes of E2 exposure in rat pelvic autonomic and sensory ganglia. Adult rats were euthanased and ganglia (pelvic ganglia, PG; dorsal root ganglia, DRG) removed. Dissociated neurons were cultured overnight, treated with E2 and processed for immunocytochemistry or immunoblotting. CREB activation was detected with an anti-phosphorylated-CREB (P-CREB) antibody and nuclear fluorescence intensity (NFI) measured. Neurites were visualised with antibodies against neuronal markers and traced. In DRG cultures E2 (10nM; 30min) increased P-CREB NFI. This was blocked by an ER antagonist. Immunoblotting showed increased phosphorylation of ERK in response to E2. E2 activation of CREB was blocked by an ERK inhibitor but not by a PI3K inhibitor. Therefore, E2 is activating CREB via ERK and not PI3K and this activation involves ERs. E2 did not activate CREB in PG cultures but increased neurite length and branching after 24hr in parasympathetic and sympathetic neurons. This was modulated by inhibiting PI3K. Effects of E2 on neurite extension in DRG are being investigated. This is novel evidence that estrogen provides neurotrophic support to neurons innervating the urogenital tract, but has different effects on subpopulations of peripheral neurons (i.e autonomic vs. sensory). This raises questions regarding the effect of changing steroid levels (e.g. ageing) on sensory signalling (e.g. pain) or degeneration and regeneration (e.g. autonomic or sensory neuropathies).

153

OESTROGEN STIMULATES HEPATIC EXPRESSION OF SUPPRESSORS OF CYTOKINE SIGNALLING-2 AND -3.

G. M. Leong [1], S. Moverare [2], N. T. Doyle [1], J. Brce [1], K. Sjogren [1,2], K. Dahlman-Wright [3], J. A. Gustafsson [3], K. K.Y. Ho [1], C. Ohlsson [2], K. C. Leung [1]

[1] Pituitary Research Unit, Garvan Institute for Medical Research, Darlinghurst, NSW, Australia

[2] Center for Bone Research, Sahlgrenska Academy, Goteborg University, Goteborg, Sweden

[3] Medical Nutrition and Bioscience Depts., Karolinska Institute, Huddinge, Sweden

The suppressors of cytokine signalling (SOCSs) are important negative regulators of cytokine action. We have recently reported that oestrogen up-regulates SOCS-2 expression, but not that of SOCS-1 and SOCS-3, to inhibit GH signalling in human embryonic kidney cells (1). In this study, we investigated whether oestrogen regulates SOCS expression in the liver, a major target tissue of GH, using in vivo and in vitro models. In vivo, the effects of 17β-oestradiol (E2) on mRNA expression of SOCS-1, -2, -3 and CIS in liver tissues from ovariectomised wild-type (WT), ERα-/-, ERβ-/- and

ERα-/-β-/- mice were examined by the microarray technique. In vitro, human hepatoma cells (HuH7) transiently expressing ERα were treated with 100nM E2 for up to 4h before measurement of SOCS-2 and SOCS-3 mRNA by quantitative RT-PCR. Furthermore, the effect of E2 on promoter activity of the murine SOCS-3 gene was studied by transfecting HuH7 cells with a luciferase reporter construct containing the mSOCS-3 promoter. E2 treatment stimulated hepatic expression of SOCS-2 and SOCS-3 by 1.5-2 fold in WT and ERβ-/- mice, but not in the ERα-/- and ERα-/-β-/- littermates. Expression of SOCS-1 and CIS was not affected. In HuH7 cells, E2 significantly increased the mRNA levels of SOCS-2 and SOCS-3 in a time-dependent manner, to maximal levels of 246±71% and 190±11% of control (mean±SE; $P<0.05$) at 2h and 1h, respectively. E2 also stimulated the SOCS-3 promoter activity to 147±8% of control ($P<0.05$). In summary, in contrast to kidney cells, E2 up-regulated the expression of both SOCS-2 and SOCS-3 in hepatic tissues. This action was mediated by ERα, and was likely through an E2-dependent transcriptional effect. We conclude that oestrogen exerts a tissue-dependent effect on SOCS expression. This may have implications for oestrogen regulation of cytokine action in different tissues.

(1) Leung KC et al. PNAS 100:1016-1021, 2003

154

TRANSFORMING GROWTH FACTOR (TGF)- B CAN REGULATE INHIBIN BINDING VIA BETAGLYCAN EXPRESSION IN MOUSE CELL LINES

Y. Wang , G. T. Ooi , J. K. Findlay , P. G. Farnworth

Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Inhibin A, a member of the TGF- β superfamily, has been proposed to inhibit the actions of activin and BMP by sequestering the type II receptors for each agonist in high affinity complexes with betaglycan [1]. Inhibins A and B abolish activin action in mouse adrenocortical (AC) cells, consistent with this model, but poorly antagonize BMP action. We previously found that BMP insensitivity to inhibin action in AC cells coincides with their selective suppression of endogenous betaglycan expression, and reduction of inhibin binding. We have therefore examined whether TGF- β isoforms, which regulate betaglycan expression in other cell types, modify betaglycan expression and inhibin binding in AC, Leydig (TM3) and Sertoli (TM4) cells. Cell cultures were treated overnight with TGF- β 1 or - β 2, after which the levels of betaglycan mRNA, corrected for GAPDH content, were measured using real-time RT-PCR, and [^{125}I]inhibin A binding was determined. In TM3 and TM4 cells, TGF- β 1 suppressed betaglycan mRNA expression, and decreased subsequent inhibin binding, at much lower concentrations than those required to compete directly for inhibin binding sites (TM3: IC50 of 60 vs. 1200 pM; TM4: IC50 of 100 vs. 1000 pM). A similar pattern was observed with TGF- β 2. In contrast, pre-treatment of AC cells with TGF- β isoforms neither modified the level of betaglycan mRNA nor shifted the dose-response curves for suppression of inhibin binding by TGF- β . The response of each cell line to TGF- β pre-treatment reflected its expression of mRNA encoding TGF- β receptor type II: expression in AC cells was negligible. [^{125}I]inhibin a ffinity labelled protein species of deduced size 115 and >170 kDa, consistent in size with betaglycan forms, were the primary targets for suppression by TGF- β in TM3 and TM4 cells. In summary, TGF- β suppression of betaglycan expression in inhibin target cells decreases inhibin binding to those cells. This should thereby reduce the effectiveness of inhibin to sequester type II receptors for activin/BMP. We conclude that the adrenocortical and gonadal effects of inhibin will be determined in part by the relative levels of extracellular TGF- β through the expression of betaglycan.

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(1) Wiater & Vale, J Biol Chem 278: 7934 (2003)

DIFFERENTIAL ACTIONS OF BAMBI ON TGFB SUPERFAMILY MEMBER SIGNALLING

R. M. Escalona [1], K. L. Loveland [2,3], M. R. Bakker [2], G. T. Ooi [1]

[1] Prince Henry's Institute of Medical Research, Clayton, VIC, Australia
[2] Monash Institute for Reproduction and Development, Clayton, VIC, Australia
[3] The ARC Centre of Excellence in Biotechnology and Development, Australia

Activins, transforming growth factors (TGFβ) and bone morphogenetic proteins (BMPs) are vital for embryogenesis, cellular development and differentiation. Their activities are mediated by type I and type II serine/threonine kinase receptors. TGF-β superfamily members signaling is controlled through an elaborate network of extracellular and intracellular regulators. The transmembrane protein BAMBI (BMP and activin membrane-bound inhibitor), first isolated in *Xenopus sp.*, was shown to inhibit BMP, TGFβ and activin signaling by forming non-signalling heterodimers with the type I receptor, thereby acting as a dominant-negative pseudoreceptor[1]. However, the low expression of BAMBI in metastatic human melanoma cell lines compared to non-metastatic cells[2] is inconsistent with the role of BAMBI as an inhibitor, since TGFβ superfamily members are strongly antiproliferative agents. We now show that rat BAMBI (rBAMBI) can both enhance and antagonise TGFβ superfamily signaling in a context-dependent manner. To assess activin interactions, two specific activin-responsive promoter constructs were used. The first contains the *Mix-2* gene activin responsive element (pAR3-lux), and the other contains three copies of the the GnRHR promoter activin-responsive sequence (pGRAS-lux). These constructs were transfected into either LβT2 mouse gonadotrope cells (pGRAS-lux) or P19 mouse embryonic carcinoma cells (pAR3-lux). Activin dose-dependently increased promoter activity (ED50 = 81 ± 25 pM), and rBAMBI increased maximal stimulation by activin to 2.3 ± 0.2 fold. In contrast, rBAMBI had no effect on BMP-4 stimulation of pVent-lux in P19 cells. These results indicate that in addition to its reported antagonistic function, BAMBI can enhance activin action, depending on target gene transcription. The mechanisms underlying these differential effects of BAMBI remain to be elucidated and we are exploring the differences between *Xenopus* and rat forms. *Supported by the NH&MRC of Australia (# RegKey 241000 #1147386, 143792)*

(1) Nature (1999) 401:480
(2) Int. J. Cancer (1996) 65:460

PROLACTIN-INDUCED PHOSPHORYLATION OF CREB IN GONADOTROPHIN-RELEASING HORMONE NEURONS IN MICE

D. R. Grattan [1], A. E. Herbison [2]

[1] Centre for Neuroendocrinology and Department of Anatomy & Structural Biology, University of Otago, Dunedin, New Zealand

[2] Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, New Zealand

Hyperprolactinaemia causes infertility, but the mechanisms involved are not known. Gonadotrophin-releasing hormone (GnRH) neurons of the hypothalamus control reproduction by inducing release of gonadotrophins from the anterior pituitary gland. Prolactin-induced suppression of reproduction is associated with a decrease in the pulsatile secretion of gonadotrophins, an effect that could be mediated by actions either in the hypothalamus or pituitary. The aim of the present study was to determine whether prolactin specifically influences the activity of GnRH neurons in the mouse brain, using phosphorylation of CREB (pCREB) as a marker for activation of various signal transduction pathways in these neurons. Adult female C57BL6/J mice were ovariectomised and treated with a subcutaneous implant containing 17β-estradiol to maintain low physiological levels of plasma estradiol. Five days later, mice were divided into three groups: ovine prolactin treatment (50 µg/injection, s.c.); bromocriptine-treatment (100 µg/injection s.c.); and saline-treated controls. Mice were treated twice daily for 48 hours (final injection 1 hour prior to sacrifice). Mice were perfused transcardially with 4% paraformaldehyde, and brains processed for double label immunohistochemistry (30 µm frozen sections). GnRH neurons (LR-1) and pCREB (Cell Signalling) were detected using specific antibodies. Eight to ten sections throughout the rostral preoptic area were analysed in each brain, and the total number of GnRH neurons expressing pCREB was counted (an average of 65-75 GnRH neurons analysed, per brain). Under control conditions expression of pCREB in GnRH neurons was low (an average of 2.2 ± 0.33%). Bromocriptine-treated animals were not significantly different (2.5 ± 1.62%). Prolactin treatment resulted in a 4-fold increase in pCREB expression, however, with an average of 9.82 ± 3.70 GnRH neurons expressing pCREB ($p<0.05$). These data provide evidence that chronic prolactin treatment induces CREB-mediated cell signalling in GnRH neurons, and are consistent with the hypothesis that prolactin inhibits reproduction through an action on GnRH neurons.

EFFECTS OF PHYTOESTROGENS ON THE OVARIAN AND PITUITARY PHENOTYPES OF ESTROGEN DEFICIENT FEMALE AROMATASE KNOCKOUT MICE

K. L. Britt [1,2], **E. R. Simpson** [1], **J. K. Findlay** [1]

[1] *Prince Henry's Institute of Medical Research, Clayton, VIC, Australia*

[2] *Monash University, Department of Biochemistry and Molecular Biology, Clayton, VIC, Australia*

Phytoestrogens can induce both estrogen agonistic and antagonistic effects, depending on the tissue, estrogen receptor content and endogenous levels of estrogen. Dietary phytoestrogens are promoted as alternatives to synthetic estrogens for hormone replacement therapy, however their effects on the reproductive axis have not been exhaustively studied in vivo. Female aromatase knockout mouse (ArKO) mice are estrogen-free, and anovulatory with a block in folliculogenesis, hemorrhagic cysts and development of Sertoli cells within their ovaries. We evaluated the ArKO mouse as a model to test the effects of phytoestrogen-supplemented diets on the reproductive organ weights, ovarian morphology, gonadotropin levels and the transcript levels of ovarian somatic cell and steroidogenic markers. The extent to which phytoestrogens either as soyfree or soymeal diet or genistein added to a soyfree diet, exert estrogenic effects varied with the type of phytoestrogen and the parameter being tested. The genistein diet significantly increased uterine and ovarian weights of ArKO compared to wildtype mice, whilst both the soy, and to a larger extent the genistein diet, improved ovarian morphology compared to the soyfree diet. Transformation to morphological Sertoli cells in ArKO mice was decreased by both phytoestrogen diets, whilst the gene expression of Sertoli cell markers was not affected. The soy diet increased both LH and FSH in both genotypes compared to animals on the soyfree diet. The genistein diet reduced FSH levels in ArKO mice, correlating with increased ovarian inhibin subunit expression. In conclusion, phytoestrogens are estrogenic in ArKO mice. Specifically, they can affect serum gonadotropin levels, and offset the development of Sertoli cells and hemorrhagic cysts within the ovaries, depending on the type of dietary phytoestrogen. Further studies are required to determine the effective doses and treatment regimes for phytoestrogens as endocrine modulators. Supported by NH&MRC Reg Key#198705

POLYCYSTIC OVARY SYNDROME: ARE ENDOCRINOLOGISTS AND GYNECOLOGISTS TREATING THE SAME PATIENTS?

A. J. Cussons [1,2], B. G.A. Stuckey [1,2], J. P. Walsh [1,2], V. Burke [3], R. J. Norman [4]

[1] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, WA, Australia
[2] Keogh Institute for Medical Research, Sir Charles Gairdner Hospital, Perth, WA, Australia
[3] School of Medicine and Pharmacology UWA, Royal Perth Hospital, Perth, WA, Australia
[4] Research Centre for Reproductive Health, The Queen Elizabeth Hospital, Adelaide, SA, Australia

Context. Women with polycystic ovary syndrome commonly consult endocrinologists or gynecologists. The diagnosis and management of this disorder are controversial, and it is not known if these specialty groups differ in their approach. Objective. To compare the investigation, diagnosis and treatment of polycystic ovary syndrome by endocrinologists and gynecologists. Design and Setting. A questionnaire containing a hypothetical patient case history with varying presentations was sent to endocrinologists and gynecologists in teaching hospitals and private practice. Results. Evaluable responses were obtained from 138 endocrinologists and 172 gynecologists. The two specialty groups differed markedly in their choice of essential diagnostic criteria. Endocrinologists regarded androgenisation (81%) and menstrual irregularity (70%) as essential for diagnosis, whereas gynecologists cited polycystic ovaries on ultrasound (61%), androgenisation (59%), menstrual irregularity (47%), and elevated LH:FSH ratio (47%). (All p values <0.001). Gynecologists were more likely to request ovarian ultrasound (91% vs 44%, p<0.001) whereas endocrinologists were more likely to measure adrenal androgens (80% vs 58%, p<0.001) and fasting lipids (67% vs 34%, p<0.001). Gynecologists were less likely to assess glucose homeostasis but were more likely to use a glucose tolerance test to do so. Diet and exercise were chosen by most respondents as first line treatment for oligomenorrhoea, hirsutism, infertility and obesity. Endocrinologists were more likely to use insulin sensitisers, particularly metformin for these indications. In particular, for infertility, endocrinologists favored metformin treatment whereas gynecologists recommended clomiphene. Conclusions. There is a lack of consensus between endocrinologists and gynecologists in the definition, diagnosis and treatment of polycystic ovary syndrome. Women may receive different diagnostic advice and treatment depending on the type of specialist consulted.

OVARIAN LEUKOCYTES AND CYTOKINES IN POLYCYSTIC OVARY SYNDROME

R. J. Norman , R. Wu , S. Fujii , N. Ryan , K. Van Der Hoek , M. Jasper , I. Sini

Obstetrics and Gynaecology, The University of Adelaide, 28 Woodville Road, WOODVILLE, SA, Australia

Introduction Polycystic Ovary Syndrome (PCOS) is associated with anovulatory infertility and metabolic disturbances. PCOS has an unknown aetiology but is involved with aberration of substances that lead to follicular growth. The ovarian leukocyte/cytokine network is important in the ovary and has not been adequately examined in PCOS. The aim of the study was to look at the distribution of leukocytes in the ovaries of women with PCOS and to look at expression of cytokine and chemokine mRNA in follicular cells from these patients. Materials and Methods Ovaries were obtained from PCOS (n=5) and non PCOS (n=4) women undergoing gynaecological surgery for non ovarian conditions prior to the menopause. They were immunostained for a wide variety of leukocyte markers and distribution counted using visual imaging software. Luteinising granulosa cells were obtained prior to ovulation in women undergoing *in vitro* fertilization with (n=11) and without (n=22) PCOS and mRNA studied using quantitative RT-PCR for various cytokines and chemokines. Results The CD45RO subset of leukocytes (principally activated/memory T-lymphocytes) were significantly decreased in ovaries from PCOS women compared to non PCOS women. The other leukocytes were not different in distribution and numbers. Transcripts for CSF-1, IL-1 b , IL-6, IL-8, IL-10, MCP-1 and TNF a were not different between PCOS and non PCOS women whilst GM-CSF mRNA was not detectable in either group. There was an association between high testosterone levels and high IL1 b and low TNF a transcripts. Women who became pregnant following IVF had higher levels of IL-10 mRNA. Conclusion The role of T-lymphocytes in PCOS needs further examination, and if the leukocyte/cytokine network in PCOS is important, other cells and cytokines need examination. This is the first study to definitively describe the leukocyte/cytokine network within polycystic ovaries. While other cells and substances may be important in PCOS and intervention procedures such as ovarian drilling, it does not appear as though macrophages, neutrophils, B-lymphocytes and a variety of cytokines are involved in the aetiology of PCOS.

MEASUREMENT OF TOTAL TESTOSTERONE IN WOMEN: COMPARISON OF A DIRECT RADIOIMMUNOASSAY VERSUS RADIOIMMUNOASSAY AFTER ORGANIC SOLVENT EXTRACTION AND CELITE PARTITION COLUMN CHROMATOGRAPHY

S. L. Davison [1], R. J. Bell [2], J. G. Montalto [3], K. Sikaris [4], S. Donath [2], F. Z. Stanczyk [5], E. R. Simpson [6], S. R. Davis [2]

[1] Biochemistry, Monash University, The Jean Hailes Foundation Research Unit, Clayton, VIC, Australia
[2] Obstetrics/Gynaecology, Monash Uni, The Jean Hailes Foundation, Clayton, VIC, Australia
[3] Biochemistry, Mayne Health Dorevitch Pathology, Heidelberg, VIC, Australia
[4] Biochemistry, Melbourne Pathology, Melbourne, VIC, Australia
[5] Obstetrics and Gynaecology, USC Keck School of Medicine, Los Angeles, United States
[6] Prince Henry's Institute of Med Res, Clayton, VIC, Australia

Background and aims: Testosterone (T) has multiple significant physiological effects in women. To date no rapid, simple assay of total T has been shown to produce reliable results in women at the low end of the normal female range. The aim of this study was to evaluate the accuracy of a direct radioimmunoassay (dRIA) for total T by comparing values for total T measured by this assay with values determined by a conventional RIA (cRIA) method that utilizes extraction and chromatographic steps prior to quantification. Methods: Fasting serum samples were obtained from a sub-group of 259 healthy women, aged 18-75 years, randomly recruited from the community and stored at -80°C. Total T was measured by the dRIA method using antibody coated tubes and iodine-labeled T tracer. For comparison, total T levels were also measured using the cRIA after organic solvent (ethylacetate:hexane (3:2)) extraction and Celite column partition chromatography prior to RIA. Results: The mean T level by dRIA was 0.76 nmol/L (median 0.70, SD 0.54, min 0.10, max 3.2). The mean difference between the two measurements (dRIA-cRIA) was –0.28 (SD 0.3). The limits of agreement using the Bland-Altman approach on log transformed data showed that, on average, the dRIA value was 63% of the cRIA value and that 95% of the time the dRIA estimate lay between 26% and 155% of the cRIA estimate. However, with respect to clinical application, for classification of values in the lowest 10th centile, agreement between assays was seen in 245/259 women (Kappa=0.68) Conclusion: The dRIA is a clinically useful assay that provides precise measurements of total T in women, particularly when values are low, and is appropriate for the study of the issue of "low" T within the female population.

EFFECT OF SEX STEROIDS ON BMD, ANTHROPOMETRY AND LIPIDS IN TRANSSEXUAL SUBJECTS.

A. M. Story [1], F. Lang [2], M. Hooper [2], C. White [1]

[1] Diabetes Endocrinology and Metabolism, Prince oF Wales Hospital, Randwick, NSW, Australia
[2] Diabetes and Endocrinology, Concord Hospital, Sydney, NSW, Australia

Sex steroids are important in the preservation of bone mineral density (BMD) in men and women. Furthermore, they maybe responsible for regional differences in body composition. In addition, men are recognised to have a two-five fold increased risk from cardiovascular disease compared to women (1) and differences in atherogenic lipid profiles may contribute to this. With this background, we analysed data from a cohort of gonadectomized M-F transsexuals on estrogen replacement and compared it with that of healthy, eugonadal control males. 19 transsexual men and 13 controls underwent testing of; BMD (lumbar spine, femoral neck and whole body), body fat composition (arm, leg, trunk, total), fasting blood sugar with insulin levels, fasting cholesterol, sex hormone binding globulin (SHBG) and testosterone. There was no significant difference between mean age, weight and BMI in the two groups. Transsexual males had a significantly lower testosterone than the eugonadal males and the effect of estrogen in this group was demonstrated by higher SHBG levels and suppressed gonadotrophin levels. The mean number of years from gonadectomy was 9 +- 9. Mean number of years of estrogen replacement pre and postoperatively were 4 +-3 and 6 +- 6 respectively. In univariate analysis the following significant results were obtained (mean of transsexual data VS mean of control data); % Leg fat: 28% VS 19%, $P < 0.001$; Total lean tissue: 47.6kg VS 53.0kg, $P = 0.01$; HDL cholesterol: 1.76mmol/L VS 1.29mmol/L, $P = 0.032$; LDL cholesterol: 2.43mmol/L VS 3.46mmol/L, $P=0.006$. The lack of significant difference in BMD at any site between the two groups was in keeping with our hypothesis that estrogen preserves bone mass in hypogonadal males. Transsexual males were leaner and had a greater deposition of fat on the upper leg consistently. Estrogen in the transsexual group was associated with a positive effect on lipids with higher HDL and lower LDL. Overall this study demonstrates that estrogen replacement in M-F transsexuals is associated with preservation of BMD, regional body composition differences and altered lipid profiles.

(1) Jousilahti et al Circulation 1999.

VARIATION OF BONE AND CONNECTIVE TISSUE TURNOVER MARKERS WITH AGE, GENDER, BMI AND ETHNICITY IN ELITE ATHLETES

A. E. Nelson [1], C. J. Howe [2], M. J. Seibel [3], T. V. Nguyen [1], J. De Winter [3], K. C. Leung [1], G. J. Trout [2], R. C. Baxter [4], D. J. Handelsman [3], M. Irie [5], R. Kazlauskas [2], K. K. Ho [1]

[1] Pituitary Research Unit, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[2] Australian Sports Drug Testing Laboratory, Pymble, NSW, Australia
[3] ANZAC Research Institute, Concord, NSW, Australia
[4] Kolling Institute of Medical Research, St Leonards, NSW, Australia
[5] TOHO University, Tokyo, Japan

The aim of this study was to determine the effect of the demographic factors age, gender, BMI and ethnicity on bone and connective tissue turnover markers in elite athletes, in order to establish reference ranges. These markers are responsive to growth hormone (GH) and remain elevated following withdrawal of exogenous GH so are potential indirect markers of GH doping. Serum samples were obtained from 1087 elite athletes aged 22 ± 5 years from 14 countries, representing the ethnic groups: Caucasian (53%), Asian (32%), African (10%) and Oceanian and other (5%). Bone and connective tissue markers were measured in duplicate by radioimmunoassay: N-terminal propeptide of type I procollagen (PINP), a marker of bone formation; N-terminal propeptide of type III procollagen (PIIINP), a marker of connective tissue collagen synthesis; C-terminal cross-linked telopeptide of type I collagen (ICTP), a marker of bone resorption. Statistical analysis was performed by random-effects analysis of variance. There was a highly significant inverse relationship between all three markers with age. All markers were significantly higher in males than in females and were negatively correlated with BMI. Multiple regression analysis indicated that age, gender, BMI and ethnicity accounted for 32-48% of the total variability of serum PINP, ICTP and PIIINP. Age exerted the greatest effect (PINP: 27%, ICTP: 42%, PIIINP: 27% of the variability), then gender (7.6%, 4.6%, 2% respectively). There was modest contribution to the variability by BMI (1.3%, 0.6% and 0.9% respectively) and ethnicity (0.7%, 0.01% and 2% respectively). In conclusion, reference ranges for the GH-sensitive bone and connective tissue markers PINP, ICTP and PIIINP should take into account demographic factors such as age and gender. For these markers, however, reference ranges can be established without needing to account for BMI and ethnicity. (Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program).

164

FRANKSTON BONE PROTECTION PROJECT: FAILURE OF INPATIENT AND OUTPATIENT MANAGEMENT OF OSTEOPOROSIS 12 MONTHS AFTER A FRAGILITY FRACTURE.

S. Varadarajan , K. Quick , D. Dutta , S. Morris , C. Gilfillan

Department of Medicine, Frankston Hospital, Peninsula Health Care Network. Victoria, Frankston, VIC, Australia

We are conducting a telephone review of patients who had sustained a low-trauma fracture over the age of 40 who were admitted to Frankston Hospital over a 6 month period in 2003. Of the 323 patients admitted to Frankston Hospital during this period, 70 (22%) were male (mean age 76) and 253 (78%) were female (mean age 79). 157 (49%) had hip fractures whereas only 11 (3%) had spinal fractures. Only 2 patients had a DEXA scan and only 9 (3%) had any osteoporosis therapy commenced by the time of hospital discharge. No patient had their vitamin D status checked. 130 of the 323 patients were contacted 12 months after discharge to see if investigation and treatment has been initiated whilst outpatients. Of the 130 contacted, 9 (6.7%) were deceased, 14 (10.7%) had been commenced on alendronate, 3 were on risedronate (2.2%) and 2 (1.5%) were on raloxifene (Selective Estrogen Receptor Modulator). 5 patients (3.7%) were on Calcium supplementation, 2 (1.5%) were on Vit D. 26 (19.8% of total) patients had undergone a DEXA scan. The remainder of 77 patients contacted (57%) were on not on any specific treatment for osteoporosis. This indicates that patients with low-trauma fractures are still not being adequately investigated or treated for osteoporosis. We aim to complete our study and to develop an in-hospital protocol to address this treatment gap.

165

SPINAL CORD COMPRESSION SECONDARY TO MARKED ANGULATION OF THE SPINE AFTER PERCUTANEOUS VERTEBROPLASTY – A CASE REPORT

C. R. Ong [1,2], F. Milat [1], A. J. Weissberger [1], J. A. Eisman [1,2], J. R. Center [1,2]

[1] Endocrinology, St Vincent's Hospital, Sydney, Darlinghurst, NSW, Australia

[2] Bone and Mineral Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

Vertebral compression fractures occur in 20% of people aged over 70 years and are associated with increased mortality and chronic pain. Percutaneous vertebroplasty is becoming increasingly used in the treatment of severe pain following vertebral fractures. An important but often overlooked sequel of vertebroplasty is the development of new vertebral fractures shortly after the procedure. We report a case of an 80-year old woman diagnosed with osteoporosis 13 years ago. Specific anti-resorptive therapies were initiated on multiple occasions but poorly tolerated. In 2002, she suffered a collapse-crush fracture of T12 and significant anterior wedging of T11. Radionuclide bone scan showed increased osteoblastic activity in T11 and T12. Marrow oedema was seen on MRI. As she was intolerant of oral bisphosphonates, intravenous pamidronate was given. Due to severe and persistent pain, percutaneous vertebroplasty at T11 was performed. This gave minimal pain relief and was followed within 2 weeks by a further L1 fracture. Shortly after this event, she developed symptoms of neurogenic bladder and bowel with overflow incontinence and severe constipation requiring manual disimpaction. MRI revealed marked angulation and increased signal intensity of the cord at the level

of the multiple adjacent fractures, consistent with mild cord compression. However, adequate space was seen posterior to the cord and neurosurgery was deemed inappropriate. This case presents an unusual cause of spinal cord compression due indirectly to osteoporotic vertebral crush fractures and highlights the potential problem of vertebral fractures adjacent to vertebrae treated by percutaneous vertebroplasty.

166

TWO CASES OF HYPERANDROGENISM IN THE SETTING OF LEFLUNOMIDE (ARAVA) THERAPY

F. Milat [1], B. G.A. Stuckey [2], H. Teede [1]

[1] Department of Endocrinology, Southern Health, Dandenong, VIC, Australia
[2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

Leflunomide (Arava) is an immunomodulatory prodrug used in the treatment of rheumatoid arthritis. The active metabolite of leflunomide inhibits de novo synthesis of pyrimidine. Alopecia occurs in approximately 10% of patients on leflunomide but hyperandrogenism has never been reported. We report two cases of severe hyperandrogenism in the setting of leflunomide therapy. Case 1: A 58yr-old woman commenced leflunomide for rheumatoid arthritis. Her other medication was metoprolol. Following 12 months of therapy, the patient noted male pattern baldness, hirsutism, deepening of the voice and increased energy. Serum testosterone was 13.3nmol/L (0.5-4.0), SHBG 34nmol/L (18-114) and FAI 39.1, oestradiol 120 pmol/L, FSH 54.2 IU/L, LH 44.2 IU/L and 17-0H progesterone levels, thyroid function tests and urinary cortisol excretion were normal. Abdominal computerised tomography (CT) and vaginal ultrasound did not demonstrate ovarian or adrenal pathology. The patient elected to continue leflunomide and started cyproterone acetate 50mg daily, with normalisation of her androgen profile within 8 weeks. Vaginal ultrasound, CT abdomen and serum androgens at three * 6-monthly intervals remained normal. Ovarian vein sampling was not performed. Case 2: A 56yr-old woman with rheumatoid arthritis commenced leflunomide in 2000. Other medications included azathioprine, prednisolone, methotrexate and Premarin. In 2004, the patient reported increasing facial hirsutism. Serum testosterone was 8.2nmol/L(<4.0), SHBG 78nmol/L (30-90) and FAI 10.5. Other biochemistry was normal. Normal imaging with marked hyperandrogenism does not exclude rare ovarian pathology, which requires venous sampling or oophorectomy. However, case 1 has demonstrated no ovarian pathology over 2 years. The mechanisms underlying alopecia with leflunomide have not been explored. Based on these limited case studies, it may be related to hyperandrogenism. This area requires further research, which is currently underway. However, in the interim all cases of hyperandrogenism with leflunomide should be reported to ADRAC.

167

OESTROGEN TREATMENT TO REDUCE THE ADULT HEIGHT OF TALL GIRLS: HOW WELL DID IT WORK?

A. J. Venn [1], T. Hosmer [1], D. Hosmer [1], F. Bruinsma [2], P. Jones [2], J. Lumley [2], P. Pyett [2], J. Rayner [2], G. Werther [3]

[1] Menzies Research Institute, Hobart, TAS, Australia
[2] Centre for the Study of Mothers' & Children's Health, Melbourne, VIC, Australia
[3] Centre for Hormone Research, Murdoch Children's Research Institute, Melbourne, VIC, Australia

Introduction: Oestrogen treatment to reduce the adult height of tall girls has been available since the 1950s. This study aimed to examine how well treatment worked. Methods: Eligible subjects were identified from the medical records of Australian paediatricians who assessed tall girls from 1959 to 1992. All subjects had an estimated mature height (EMH) prediction using the method of Bayley and Pinneau: they included girls who received oestrogen treatment in adolescence (treated group) and those who did not (untreated group). Data on EMH predictions were abstracted from medical records. Women self-reported their final adult height in a follow-up questionnaire. The difference between the last EMH prediction (prior to treatment in the treated group) and the final adult height was calculated. Results: The sample included 279 treated women and 367 untreated women. The mean EMH prediction at last assessment (181.4cm treated, 174.6cm untreated) and mean final adult height (179.3cm treated, 176.5cm untreated) were significantly greater in the treated than untreated groups ($p<0.01$). The mean difference between the EMH and final height was 2.13cm in the treated group and -1.86cm in the untreated group. A fitted linear regression model showed that the significant predictors of the height difference, in addition to treatment, were skeletal age, height and breast Tanner stage at last EMH prediction, and the age at menarche. Using this model, for a mean breast Tanner stage of 3.4 and mean skeletal age of 12.8 years at the time of last EMH assessment, oestrogen treatment reduced height by 3.5cm. Height reduction was greater for those who commenced treatment at an earlier skeletal age and Tanner stage. Conclusions: On average, oestrogen treatment in tall girls resulted in an adult height that was less than predicted. The effect was greatest in those who commenced treatment at an earlier stage of maturity.

168

QUANTIFYING LONG-RANGE CORRELATION PROPERTIES OF GLYCEMIC CONTROL - METHOD AND PHYSIOLOGICAL IMPACTS.

U. Meinhardt [1], P. E. Mullis [1], D. Baldwin [2], U. Frey [2]

[1] University Children's Hospital, Division of Pediatric Endocrinology and Diabetology, Bern, Switzerland
[2] University Children's Hospital, Division of Pediatric Respiratory Medicine, Bern, Switzerland

Healthy systems in physiology and medicine are remarkable for their structural variability and dynamical complexity. For cardiac regulation, perturbation of the control mechanisms by disease or aging may lead to a breakdown of these long-range correlations that normally extend over thousands of heartbeats. The detrended fluctuation analysis (DFA) is based on non-linear system theory. The calculated α value characterizes the correlation properties of the original time series. For a random process, α takes the value of 0.5. For a positively correlated signal (large fluctuations are likely to be followed by large fluctuations), α is >0.5. The aim of this study was to determine, whether integrative

glucose regulation is correlated to the glucose value in the past and whether it can be characterized by DFA. Furthermore in case of correlation we were interested in a) reproducibility, b) patterns in glucose control during day- and nighttime, and c) patterns in healthy controls versus Type 1 Diabetes Mellitus (T1DM) under continuous subcutaneous insulin infusion therapy (CSII). To measure glucose fluctuations we used the continuous glucose monitoring system (CGMS)®, which generated 800 data points in 5 minutes intervals. These were analyzed by DFA. We measured 12 and 15 time series in young healthy adults (mean age 27.3 years) and adolescent T1DM CSII patients (mean age 13.7 years). In controls the α-value was 1.30 (95% confidence interval (CI) 0.04) indicating a strong correlation, taken a calculated randomized surrogate α-value of 0.55, CI 0.02. Repetitive measurements in the same individual showed clear reproducibility with similar α-values. We observed stronger long range correlation at night- than at daytime ($p = 0.014$). Finally, α-values were significantly higher in CSII patients injecting rapid-acting human insulin analoga (1.53; CI 0.05) than in controls (1.30; CI 0.04; $p < 0.001$), which is consistent with a stronger regulation of the control loop. In conclusion we found evidence of long range correlation ("memory") in overall glucose regulation. Memory of glucose control can be characterized by a single parameter α using DFA and CGMS® and is significantly different in T1DM under CSII. In future studies α can potentially be used for diagnostics or monitoring diabetic control.

169

DETERMINANTS OF TESTICULAR FUNCTION AND BONE DENSITY IN PARKINSON'S DISEASE.

L. A. Turner [1,4], M. Schipper [1,4], A. Conway [1,4], L. P. Ly [1,4], C. Meier [2,4], M. Seibel [2,4], S. Mercer [3], M. Hayes [3], D. J. Handelsman [1,4]

[1] Andrology Dept, Concord Hospital, Concord, NSW, Australia
[2] Endocrinology Dept, Concord Hospital, Australia
[3] Neurology Dept, Concord Hospital, Australia
[4] ANZAC Research Institute, Australia

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder featuring tremor, rigidity and akinesia, which lead to a profound movement disorder in older people. Recent studies suggest testosterone may improve non-motor symptoms (Okun et al Arch Neurol 59:1750-3, 2002). We therefore studied 50 men with PD (age 69 ± 1 yr, range 42-89; BMI 25.6 ± 0.4 kg/m^2) to characterize their androgen status, bone density and relationship to markers of disease stage, severity and clinical features. Patients were classified into Hoehn & Yahr (HY) disability stages 1, 2 & 3 (n=14, 26, 10, respectively) with functional evaluation according to the Unified PD Rating Scale (UPDRS) part II (daily living activities, DLA) and III (motor function, MF), lower urinary tract symptoms (IPSS) and general quality of life (SF36). Reproductive hormones (LH, FSH, total and free T, SHBG, estradiol, prolactin) were measured by immunoassay, bone mineral density (BMD) by DEXA at hip and spine with body composition by bio-impedance analysis. As expected, progressively worse PD according to HY stage was significa ntly associated with older age (63±2, 70±2, 76±2), worse functional status (DLA 9±1, 15±1, 24±1; MF 16±1, 31±2, 44±2, respectively) and quality of life (4 of eight SF36 domains) and reduced hand-grip dynamometry (37±2, 31±2, 25±2) but lower urinary tract symptoms were not related to stage. There was a significant decrease in mean testis volume (19 ± 1, 20 ± 1, 15 ± 2, $p=0.03$) but no change in hormone status (blood LH, FSH, total and free testosterone, estradiol, prolactin) according to PD stage (blood total testosterone <10 nM in 9 and

<8 nM in 4 men) apart from an increase of marginal significance (p=0.05) in SHBG (37±5, 53±5, 60±9, respectively). Bone mineral density was found in the osteopenic (<2 SDs, n=5) or osteoporotic (<2.5 SD's, n=3) range, but there was no significant difference in spinal or hip BMD or body composition (lean or fat mass) according to PD stage. We conclude that the stage and severity do not predict reproductive hormone status, bone density or body composition in men with PD but the influence of other PD clinical features remain to be clarified.

170

DIFFERENTIAL MODULATION OF CELL CYCLE PROGRESSION AND APOPTOSIS BY SYNTHETIC AND ENDOGENOUS PPAR GAMMA LIGANDS IN MONOCYTIC CELLS.

J. K.A. Hampel [1,2], L. M. Brownrigg [1], D. Vignarajah [1], K. D. Croft [1], A. M. Dharmarajan [3], J. M. Bentel [4], I. B. Puddey [1], B. B. Yeap [1]

[1] School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
[2] Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia
[3] School of Anatomy and Human Biology, University of Western Australia, Perth, WA, Australia
[4] Department of Anatomical Pathology, Royal Perth Hospital, Perth, WA, Australia

INTRODUCTION: Thiazolidinediones (TZDs), synthetic agonists for the peroxisome proliferator-activated receptor gamma (PPARg), increase insulin sensitivity to improve glycemic control in patients with Type 2 diabetes mellitus. PPARg is expressed in macrophages within atherosclerotic plaques where it modulates lipid accumulation and cholesterol efflux. AIMS: To clarify effects of different PPARg ligands on proliferation, cell cycle, gene expression and lipid accumulation in a monocyte/macrophage cell line. METHODS: PPARg +ve U937 monocytic cells were treated with TZD ciglitazone, and natural fatty acid PPARg ligands 9- and 13-hydroxyoctadecadienoic acid (HODE). Proliferation and cell cycle progression were assessed. Apoptosis was measured by DNA quantitation, Annexin V staining and 3'end labelling. PPARg gene expression was measured by semi-quantitative reverse transcription PCR, and fatty acid content by gas chromatography. RESULTS: Ciglitazone and 9-HODE reduced U937 proliferation to 68+6% and 62+5% of control at 48 hours (both P<0.01). Ciglitazone increased cells in S phase from 46.5+2.6% to 48.2+1.8% (P=0.04), and decreased G2/M from 9.8+1.5% to 7.1+1.2% (P=0.03), suggesting inhibition of S/G2 transit. Similar effects occurred with 13-HODE. 9-HODE increased G1/0 from 43.2+3.0% to 58.2+4.0% (P=0.02), reduced S from 46.3+1.7% to 36.2+6.9% (P=0.03) and reduced G2/M from 10.6+1.4% to 5.6+3.5% (P<0.001), implying distinct effects on cell cycle progression compared with 13-HODE or ciglitazone. 9-HODE selectively induced apoptosis in U937 cells with 3.7+3.0% of apoptotic cells compared to control levels 0.2+0.2% after 24 hours (P=0.005). 9-HODE increased PPARg2 gene expression four-fold (P<0.01). All three agonists increased cellular content of C18 fatty acids. CONCLUSIONS: Synthetic and endogenous ligands for PPARg, ciglitazone and 9- and 13-HODE, possess differential, ligand specific actions in monocytic cells to regulate proliferation, cell cycle progression, apoptosis and PPARg gene expression. As HODEs are components of fatty acids derived from oxidised low-density lipoproteins, TZDs and natural fatty acid ligands for PPARg may exhibit distinct effects relevant to atherogenesis.

171

SIGNAL TRANSDUCTION PATHWAY INVOLVED IN FREE FATTY ACID-INDUCED REDUCTION OF VOLTAGE-GATED K^+ CURRENTS IN PRIMARY CULTURED RAT PANCREATIC B-CELLS

D. Feng , C. Chen

Prince Henrys Institute, Clayton, VIC, Australia

Type 2 diabetes is a disease which is tightly linked with an imbalance of energy metabolism and excessive fat tissue or obesity. Free fatty acids (FFAs) from adipocytes are an important energy source and a key regulator, besides glucose, of insulin secretion. FFAs have recently been demonstrated to be a specific ligand of an orphan G-protein-coupled receptor, GPR40, which is abundantly expressed in the pancreatic islet cells. FFAs stimulate insulin secretion from pancreatic islet cells although the mechanism involved is unclear. In this study, we investigated the in vitro effect of linoleic acid on voltage-gated K^+ currents in primary cultured rat pancreatic β-cells and the involvement of cAMP-protein kinase A (PKA) and C (PKC) systems. Nystatin-perforated whole-cell configuration was employed to record voltage-gated K^+ currents. Intracellular cAMP was measured by radioimmunoassay using the MIN6 mouse β-cell line. Linoleic acid significantly, and reversibly, decreased the amplitude of both transient and delayed rectifying voltage-gated K^+ currents, with a predominant decline in the delayed rectifying K^+ current. The linoleic acid-induced reduction in K^+ currents was abolished by the PKA inhibitor H89 and a membrane permeable cAMP antagonist Rp-cAMP in the extracellular bath solution. Linoleic acid alone had no significant effect on cAMP levels whereas a significant increase in accumulated cAMP occurred by combined linoleic acid and IBMX (phosphodiesterase inhibitor) treatment or linoleic acid and forskolin (adenocyclase stimulator) treatments . Chelerythrine (PKC inhibitor) had no effect on the K^+ current response to linoleic acid. These results indicate that FFAs reduce the voltage-gated K^+ current in rat pancreatic β-cells through an increase in cAMP production leading to activation of the PKA system. The PKC system is not involved in the K^+ current response. The reduction in voltage-gated K^+ currents may enhance Ca^{2+} influx, with membrane depolarization leading to insulin secretion from the β-cells.

172

REGULATION OF GLUCONEOGENIC ENZYMES BY GLUCOCORTICOIDS IN GLUCOCORTICOID RECEPTOR-NULL MICE

K. Tan , D. A. Bird , T. J. Cole

Biochemistry & Molecular Biology, University of Melbourne, Parkville, VIC, Australia

Glucocorticoids (GCs) are important for maintaining metabolic homeostasis and responding to stress. In particular, glucocorticoid together with insulin, adrenalin and glucagon tightly control blood glucose levels in the body. During times of stress such as starvation and intense exercise, glucocorticoids maintain and increase blood glucose levels by inhibiting the utilisation of glucose by peripheral tissues and by increasing the level of hepatic gluconeogenesis. Glucocorticoids exert the majority of their diverse effects by binding to the glucocorticoid receptor (GR). When bound by glucocorticoid, GR translocates into the nucleus and modulates the transcription rate of target genes. Previous studies have shown that GCs enhance gluconeogenesis by increasing the transcription of

gluconeogenic genes such as tyrosine aminotransferase (TAT), phosphoenolpyruvate (PEPCK), serine dehydrogenase (SDH) and glucose-6-phosphatase (G6Pase).
In this study, we have investigated the role of GR in the regulation of gluconeogenetic genes by GCs using GR-null mice that contain a gene-targeted null mutation of the GR gene. The synthetic glucocorticoid dexamethasone (DEX) was injected in adult mice and mRNA levels for selected genes measured three hours after DEX treatment by northern blot analysis. Preliminary results reveal that the mRNA levels of TAT, PEPCK and SDH are strongly induced by DEX in normal control mice but not in GR-null mice, indicating that GR is essential for GCs to regulate these genes. Expression of G6Pase however was unaffected by a three hour DEX treatment in both normal and GR-null mice. Other hepatic genes involved in gluconeogenesis to be studied and known to show regulation by GCs include alanine aminotransferase, fructose 2,6- biphosphate, and tryptophan oxygenase. Finally, subtraction PCR has been used to screen for genes that are differentially regulated by DEX in normal mice compared to GR-null mice.

173

ALTERED GENE EXPRESSION IN PANCREATIC ISLETS OF HUMANS WITH TYPE 2 DIABETES MELLITUS

J. E. Gunton [1], W. J. Hawthorne [2], Y. H. Tseng [1], C. Ricordi [3], P. J. O'Connell [2], C. R. Kahn [1]

[1] Kahn Laboratory, Joslin Diabetes Center, Harvard Medical School, Boston, United States
[2] National Pancreas Transplant Unit, University of Sydney, Westmead Hospital, Sydney, NSW, Australia
[3] University of Miami Diabetes Research In, University of Miami, Miami, United States

Background: Type 2 diabetes (T2DM) is characterized by insulin resistance and disordered β-cell function, with a particular defect in glucose stimulated insulin secretion. Specific gene mutations have been identified which lead mature onset diabetes of the young (MODY), however in most cases, the factors leading to impaired insulin secretion remain unknown. Important roles for genes involved in insulin-signalling and glucose-metabolism have been demonstrated in animal models. Human data are lacking, due to difficulty accessing pancreatic tissue and the small contribution of islets to total pancreatic mass. Methods: Gene expression in human pancreatic islets isolated from the pancreata of 7 normal and 5 T2DM subjects was assessed using oligonucleotide microarrays and real-time PCR to confirm array results. T2DM subjects were <10 y post-diagnosis. Mean age was 48 in both groups. BMI was 30.9±8.6 in controls, and 26.6±0.9 in T2DM (p=ns). Results: Amylase, glucagon, pancreatic polypeptide and somatostatin expression did not differ between groups, indicating equivalent islet purification. T2DM islets had significantly reduced expression of HNF4α (18% of control, p=0.002) , insulin receptor, (23%, p=0.027), insulin receptor substrate-2 (IRS-2), (9% of control, p=0.0001) Akt2, (16%, p=0.001) and several enzymes of the glycolytic pathway: glucose-6-phosphate isomerase, phosphofructokinase, aldolaseB, and phosphoglucomutase (all p<0.01). There was also increased expression of the PIP_3 phosphatase, SHIP2 (43% increase, p=0.013). Conclusions: Islets from humans with type 2 diabetes exhibited decreased expression of HNF4α (the MODY1 gene) and marked decreases of several key genes in the insulin-signalling and glucose-metabolic pathways. These changes would be expected to contribute to impaired glucose-sensing, β-cell function, β-cell survival and to the pathogenesis of human type 2 diabetes.

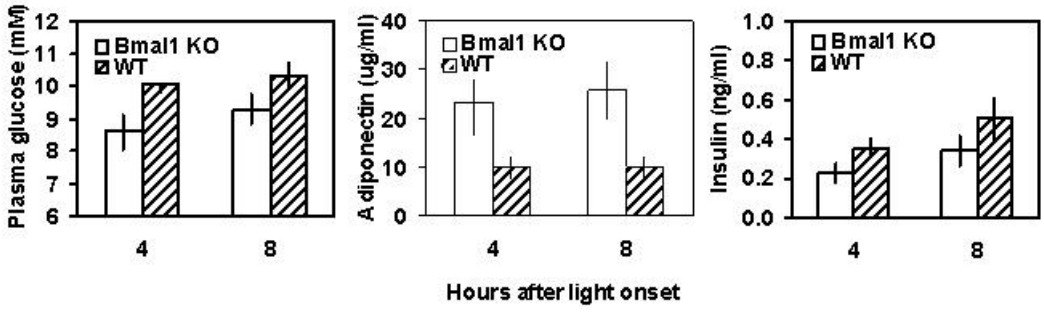
174

DISRUPTION OF THE CLOCK GENE *BMAL*1 ALTERS GLYCAEMIA AND CIRCULATING ADIPOCYTOKINES IN MICE AND INDUCES HYPOINSULINAEMIA IN MALES

D. J. Kennaway , M. J. Boden , M. DeBlasio , R. Taylor , J. A. Owens

Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, SA, Australia

The capacity to generate biological rhythmicity and entrain to the environment is now recognised as a property of all cells throughout the body. There are at least 7 critical "core" genes involved in cellular rhythmicity, including *Clock* and *Bmal* 1. The CLOCK/BMAL1 heterodimer complex provides positive drive for cellular rhythmicity while also being the main component of the output system as an inducer of functional genes. These include key metabolic genes such as glucokinase, phosphoenolpyruvate carboxykinase 1 and fatty acid synthetase, which exhibit rhythmicity in the liver. We therefore hypothesised that disruption of clock gene function will alter metabolic and endocrine functions. Wild type and *Bmal*1 knock out male (n= 8; n= 6 respectively) and female (n= 6; n= 9 respectively) mice were killed at 6 months of age, 4 or 8 hours after lights on and plasma metabolites, adipocytokines and insulin measured. The *Bmal*1 knock out mice exhibited hypoglycaemia ($p=0.002$), and increased plasma adiponectin ($p<0.0001$), particularly in females ($p<0.0001$), suggesting insulin sensitivity may be increased. Despite this, the lack of BMAL1 protein induced hypoinsulinaemia overall ($p=0.02$) and in males only ($p=0.015$). The *Bmal*1 knock out mice had increased plasma leptin ($p<0.0001$), but in males only ($p=0.001$). Plasma free fatty acids were altered in a sex specific pattern ($p=0.009$), with increased levels in knockout males, but decreased levels in knock out females. Therefore the absence of BMAL1 appears to profoundly alter circulating metabolites and related hormones, in part in a sex-specific manner, inducing an adipocytokine and FFA profile in blood consistent with leanness in females and obesity in males. With ageing, these consequences of *Bmal*1 disruption in males may lead to impaired insulin sensitivity, secretion and loss of glucose tolerance. The lack of BMAL1 also induced hypoinsulinaemia in the males, which given their increased adiponectin suggests an insulin secretory defect is already present, and would accelerate any progression to impaired insulin action and glucose tolerance. This study is the first to demonstrate a critical role of clock genes in metabolic homeostasis. The findings suggest a novel potential path to insulin resistance that may have relevance for shiftworkers experiencing prolonged disrupted rhythmicity.



ADIPOSE TISSUE PRODUCTION OF SICAM-1 IN A MURINE MODEL OF OBESITY: GENDER DIFFERENCES IN RESPONSE TO A HIGH FAT DIET

R. L. Robker [1], E. O. Smith [2], H. J. Mersmann [2], C. W. Smith [2]

[1] Research Centre for Reproductive Health, University of Adelaide, Adelaide, Texas, Australia
[2] Department of Pediatrics, Baylor College of Medicine, Houston, Texas, United States

Intercellular Adhesion Molecule-1 (ICAM-1/CD54), a transmembrane adhesion receptor that mediates immune cell migration and activation, also exists as a circulating soluble form (sICAM-1) that is increased with chronic inflammation. sICAM-1 levels are also elevated in human obesity and are positively correlated with abdominal fat deposition. We sought to analyze adipose tissue production and transcriptional regulation of ICAM-1 in a mouse model of diet induced obesity. Mice on a long term (6 months) high fat diet had elevated circulating sICAM-1 levels that positively correlated with body and abdominal fat pad weight. Northern blot analysis showed that adipose tissue expressed ICAM-1 mRNA with significantly higher levels detected in males compared to females. The cellular source of ICAM-1 was investigated by separating adipocytes and stromal-vascular (S/V) cell populations. Both fractions expressed ICAM-1mRNA however transmembrane ICAM-1 was detected only on S/V cells. sICAM-1 was produced by both adipocytes and S/V with cells from female mice secreting more than males. A short term high fat diet (3 weeks) also caused significant elevations in circulating sICAM-1 levels that correlated positively with abdominal fat pad weight. The increase in sICAM-1 was concurrent with elevations of ICAM-1 mRNA in adipose tissue. Thus, ICAM-1, produced by adipose tissue and transcriptionally regulated in response to a high fat diet, may represent a potential mechanistic link between excess abdominal fat and its associated inflammatory-like complications.

REGULATION OF CONNECTIVE TISSUE GROWTH FACTOR IN ADIPOSE TISSUE BY THE METABOLIC SYNDROME AND THIAZOLIDENEDIONE

L. Lo [2], X. Wang [2], P. F. Williams [1], J. Tan [2], F. McDougall [3], S. S.Y. Chan [3], J. Proietto [4], J. D. Zajac [4], S. V. McLennan [1,2], S. M. Twigg [1,2]

[1] Dept of Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW, Australia
[2] Discipline of Medicine, University of Sydney, Sydney, NSW, Australia
[3] Kolling Institute of Medical Research, University of Sydney, Sydney, NSW, Australia
[4] Dept of Medicine, Austin Health, University of Melbourne, Melbourne, VIC, Australia

The multifunctional protein, connective tissue growth factor (CTGF) has not been studied in adipose tissue (AdipT) or in the metabolic syndrome (MetS). A genetically induced rodent model of MetS involving over-expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene was examined, and compared with wild type age matched (4 months) PVG/c Hooded Wistars as controls. Animals (n=5/6 per group) were treated with vehicle alone or the insulin sensitising agent, pioglitazone (pio), orally 20 mg/kg/d, for 28d. Intravenous insulin tolerance tests (ITT) were then performed and tissues were isolated at termination. Epididymal (Epi) fat data are shown.

PEPCK animals were heavier than controls, and pio increased their insulin sensitivity, as well as markers of fat cell differentiation (not shown). Intact CTGF protein in tissue lysates by Western analysis was upregulated by pio. A marked increase in relative amount of a 20kDa C-terminal CTGF fragment in the MetS, was down-regulated by pio. CTGF mRNA at real-time RT-PCR was increased by MetS and was up-regulated by Pio. CTGF in subcutaneous fat was not regulated by MetS, and was increased by Pio (not shown). This data suggests that in MetS, CTGF is proteolysed in Epi, and the increases in CTGF mRNA in MetS may be counter-regulatory. Pio upregulates CTGF. Further studies are required to determine the significance of these CTGF changes in differing body fat depots. Supported by a competitive grant from Eli Lilly.

Animal group	Body weight (g)	ITT serum glucose mean fall AUC(mM)	CTGF intact protein (% control)	CTGF fragment:intact ratio (% control)	CTGF mRNA (% control)
PVG/c+vehicle	374±5.4	1.67±0.34	100	100	100
PVG/c+pio	373±5.0	2.41±0.52	194±48^{x}	86±20.1	215±36^{x}
PEPCK+vehicle	411±12.0^{i}	1.47±0.25	81±31	146±20.0^{i}	265±28^{x}
PEPCK+pio	450±5.4*^{i}	2.51±0.48*	158±3*	68±12.4*^{i}	193±25*^{x}

Data are mean±SEM *P<0.05 vs PEPCK+vehicle; ^{i}P<0.05 vs each PVG/c group;
^{x}P<0.05 vs PVG/c+vehicle group. All analysis by multiple ANOVA

PLASMA LEPTIN-BINDING ACTIVITY INCREASES FROM PRE-PUBERTY TO ADULTHOOD IN MALE RATS.

P. J. Mark [1], J. T. Smith [1,2], S. Hisheh [1], B. J. Waddell [1]

[1] Anatomy and Human Biology, The University of Western Australia, Crawley, WA, Australia
[2] Physiology and Biophysics, University of Washington, Seattle, Washington, United States

Leptin, the product of the *ob* gene, is produced primarily by adipocytes and acts via the hypothalamus to regulate food intake and energy expenditure. Leptin is also implicated as a metabolic signal for the initiation of puberty, since *ob* gene mutations prevent attainment of sexual maturity in mice. Leptin receptors exist in several isoforms (Ob-Ra to Ob-Rf), among which a soluble form (Ob-Re) binds plasma leptin and appears to restrict its access to target tissues. We have previously shown that in male rats, plasma leptin increases between days 30 and 50, and this peripubertal change is associated with an increase in hypothalamic Ob-Rb expression. The present study assessed whether plasma leptin-binding activity, presumably reflecting circulating Ob-Re, also changes over the peripubertal period and into adulthood. Plasma leptin-binding activity was measured in plasma obtained from male rats at days 30 and 50 and at 7 months. Activity increased more than three-fold from pre- to post-puberty ($P<0.05$) and then by a further two fold from post-puberty to 7 months ($P<0.05$). Because plasma leptin-binding activity is likely attributable to circulating Ob-Re, quantitative real-time PCR was used to screen several tissues for developmental changes in Ob-Re mRNA expression. This analysis demonstrated a major increase (>300-fold) in spleen Ob-Re expression between day 50 and 7 months of age; there was no change in liver, testes, epididymis or adrenal. These data suggest that the spleen accounts for at least part of the observed increase in plasma leptin-binding activity in male rats; cleavage of membrane-bound longer forms of the leptin receptor, as occurs in the human, may also contribute to this developmental change. Regardless of its source, increased plasma leptin over development is likely to reduce the metabolic clearance of leptin as occurs in rat pregnancy and limit access of leptin to Ob-Rb in target tissues.

EXPRESSION PATTERNS OF HYALURONAN, HYALURONAN SYNTHASES AND HYALURONIDASES SUGGEST A ROLE FOR HYALURONAN IN THE PROGRESSION OF ENDOMETRIAL CANCER

P. Paiva [1,2], M. P. Van Damme [2], M. T. Tellbach [1,2], T. Jobling [3], L. A. Salamonsen [1]

[1] Prince Henry's Institute of Medical Research, Monash University, Clayton, VIC, Australia
[2] Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia
[3] Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia

The extracellular glycosaminoglycan hyaluronan (HA) and its degradative enzyme, hyaluronidase (Hyal), have been proposed to play important roles in tumour metastasis and angiogenesis. HA promotes tumour cell adhesion and migration while its smaller fragments, the products of its degradation by hyaluronidase, stimulate angiogenesis. Little is known of its synthetic enzymes, the HA synthases (HAS) in cancer. HA has been recently identified in the human endometrium, where its levels vary substantially during the menstrual cycle. The aims of this study were to assess the levels of HA and how it might be regulated in endometrial cancer. Endometrial carcinomas were grouped according to histologic grade (Grade 1-3). HA histochemistry was performed using a biotinylated HA binding peptide (n=16). HAS expression was examined by immunohistochemistry using an antibody against all HAS isoforms (HAS 1, HAS 2 and HAS 3) (n=25). Real-time RT-PCR was used to determine the mRNA expression of Hyal 1 and Hyal 2 in endometrial cancers (n=15). HA, HAS, Hyal 1 and Hyal 2 were identified in endometrial carcinomas of all histologic grades. HA was predominantly localised to tumour-associated stroma. Semi-quantitative analysis revealed that HA levels correlated with endometrial cancer grade being higher in Grade 2 and Grade 3 ($p<0.05$) compared to Grade 1 carcinomas. By contrast, HAS was predominantly localised to tumour epithelial cells and its levels did not vary with tumour grade. Interestingly, the expression of Hyal 2 was substantially greater than that of Hyal 1, the major hyaluronidase type expressed in other cancers. However, the expression of neither hyaluronidase type varied with tumour grade. This is the first study to demonstrate the presence of HA, HAS, Hyal 1 and Hyal 2 in endometrial cancer. The results suggest a role for elevated HA in endometrial cancer progression justifying the need for further investigation.

179

CO-LOCALISATION OF THE PROGESTERONE RECEPTOR WITH BRCA1 IN HUMAN BREAST CANCER CELLS

K. A. Avery [1], J. D. Graham [1], A. DeFazio [1,2], C. L. Clarke [1]

[1] Westmead Institute for Cancer Research, University of Sydney at the Westmead Millennium Institute, Westmead, NSW, Australia

[2] Department of Gynaecological Oncology, University of Sydney at the Westmead Millennium Institute, Westmead, NSW, Australia

The ovarian hormone progesterone, acting through its cognate receptor, is essential for the proper development and functioning of the normal breast. However, progesterone is also known to increase the risk of developing breast cancer. The progesterone receptor (PR) exists as two isoforms PR-A and PR-B, whose expression is disrupted in breast cancer and may be associated with disease progression. Susceptibility to breast cancer is substantially increased in women who carry a mutation in BRCA1. BRCA1 is known to function as a tumour suppressor. This property is confined to endocrine tissues, even though BRCA1 is ubiquitously expressed. We have previously shown that PR isoform expression is disrupted in the normal breast of women harbouring a mutation in BRCA1. Furthermore, we have demonstrated that BRCA1 can modulate the transcriptional activity of PR in breast cancer cells, indicating that deregulation of progesterone signalling due to a mutation in BRCA1 may contribute to tumour initiation or progression. The aim of this study was to further characterise these findings by determining whether PR and BRCA1 could co-localise in hormone sensitive cells. PR-positive T-47D breast cancer cells were treated with progestin or vehicle and co-stained for either PR-A or PR-B and BRCA1. The expression of each protein was examined using immunofluorescent confocal microscopy. PR and BRCA1 were both located in the nucleus. PR-A and PR-B located to foci which we have previously shown are related to ligand binding of receptor. BRCA1 also localised to foci and current indications are that BRCA1 co-locates with both PR-A and PR-B. Current studies are aimed at determining the role of the ligand in PR-BRCA1 interactions. The demonstration that PR transcriptional activity can be modulated by BRCA1 and that PR and BRCA1 co-localise suggests a functional relationship between these proteins that may be important in breast cancer cells.

180

NF-KB SIGNALLING IN GRANULOSA CELL TUMOURS

S. Chu [1,2], P. J. Fuller [1,2]

[1] Endocrine Genetics Unit, Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

[2] Department of Medicine, Monash University, Clayton, VIC, Australia

Granulosa cell tumours of the ovary (GCT) represent ~5% of all malignant ovarian tumours. The pattern of gene expression in GCT is similar to that of FSH-dependent proliferating granulosa cells (1). FSH has been reported to stimulate the expression of X-linked inhibitor of apoptosis (XIAP) through activation of NF-κB in granulosa cells. We previously reported that the NF-κB signalling pathway is constitutively activated in two GCT-derived lines KGN and COV434. Thus the aim of this study is to ascertain the basis for this constitutive activity by detailing the gene expression profile of members of the NF-κB family in KGN and COV434 and to assess whether a similar pattern is observed in GCT. The patterns of gene expression were determined for the two cell lines, nine GCT, and nine normal premenopausal ovaries (NOv). Semi-quantitative RT-PCR assays were designed for

p50 and p65 subunits of NF-κB as well as XIAP. To investigate differential gene expression between the cell lines, GCT and NOv, we used a human NF-κB signalling pathway gene array (Superarray Q-Series GEArray). Radiolabelled cDNA from GCT, COV434, KGN and NOv were hybridized to the gene arrays. RT-PCR determinations of the p65 and p50 subunits of NF-κB and XIAP demonstrated upregulation of these genes in the GCT as compared to NOv. COV434 and KGN cells also express the p50 and p65 subunits at levels comparable to GCT, but have lower expression of XIAP than NOv. The macroarray analysis demonstrated that all groups express TNFα , with the highest levels in the NOv. A number of genes are expressed in NOv but not in the other three groups. A single exception is the interleukin-1 receptor-associated kinase (IRAK) whose expression is prominent in the cell lines and GCT whereas a low level is observed in NOv. IRAK is involved in interleukin-1 binding to its receptor to promote NF-κB and MAPK activation. Expression of IRAK has not previously been identified in either normal or malignant granulosa cells. This overexpression of IRAK may be relevant to the constitute activation of both the NF-κB and AP-1 pathways seen in the GCT-lines (2).

(1) Chu S et al Mol Endo (in press)
(2) Chu S et al Mol Hum Reprod 2002; 8(5) 426-33

181

ROLE OF THE HU PROTEINS, HUR AND HUD, IN THE REGULATION OF ANDROGEN RECEPTOR EXPRESSION AND ACTIVITY IN PROSTATE CANCER CELLS.

C. F. Down [1,2], R. R. Lareu [1,2], B. Granath [1,2], D. J. Beveridge [1,2], H. Furneaux [3], D. C. Voon [1,2], P. J. Leedman [1,2]

[1] Centre for Medical Research, WAIMR, University of WA, Perth, WA, Australia
[2] School of Medicine and Pharmacology, University of WA, Perth, WA, Australia
[3] Department of Physiology, University of Connecticut Health Centre, Farmington, Connecticut, United States

The primary treatment for prostate cancer (PCa) involves androgen ablation, halting tumour growth through down-regulation of important proliferative genes under control of the androgen receptor (AR) (1). Frequently, however, PCa progresses to an androgen-independent state and untreatable disease. Recent data indicates that the AR continues to be expressed in many of these tumors, often associated with androgen-independent activation of the AR signalling pathway. Thus, understanding the mechanisms that regulate AR expression in these cells is an important goal. We have previously shown that mRNA decay plays a critical role in AR regulation in PCa cells. Moreover, we identified a specific, UC-rich region in the 3' untranslated region (UTR) of the AR mRNA that binds, both *in vitro* and *in vivo*, HuR and HuD, members of the Hu family of RNA-binding proteins known to modulate mRNA turnover in other systems (2, 3). Significantly, HuD, which is usually restricted to neurons, is expressed in a range of primary Pca samples. In these studies, we aimed to determine the functional role of HuR and HuD in the regulation of AR expression and activity in PCa cells. We found, in cells with levels of HuR reduced by RNAi treatment, that AR mRNA and protein levels were significantly decreased. In addition, overexpression of HuR or HuD in LNCaP cells regulated AR-mediated transcription of a PSA-luciferase reporter. Taken together, these data implicate the Hu proteins, HuR and HuD, as novel AR mRNA-binding proteins that play an important role as regulators of AR expression and signalling in PCa cells. As such they may represent novel potential therapeutic targets.

Further experiments are underway to evaluate the effects of these proteins on PCa cell proliferation and growth.

(1) Culig Z, et al. Endocrine Related Cancer, 2002. 9:155-70
(2) Yeap BB, et al. Journal of Biological Chemistry, 2002. 277: 27183-92
(3) de Silanes IL, et al. Oncogene. 22: 7146-54

182

ROLES FOR GHRELIN IN PROMOTING ENDOMETRIAL ADENOCARCINOMA

N. Tawadros [1,2], L. A. Salamonsen [1], C. Chen [1,2]

[1] Prince Henrys Institute, Clayton, VIC, Australia
[2] Department of Physiology, Monash University, Clayton, VIC, Australia

The growth hormone secretagogue receptor (GHS-R) and its ligand ghrelin, are widely expressed in peripheral tissues outside the hypothalamus-pituitary axis and in several neoplastic tissues. We previously demonstrated mRNA/protein for ghrelin and GHS-R in normal human endometrium and in endometrial adenocarcinoma (1,2). Endometrial cancer is the most common gynaecological malignancy of the female reproductive tract. This study investigated whether GHS-R and ghrelin can be immunolocalised in endometrial cancer cell lines; Ishikawa, HEC1A, AN3CA, LDN (3) and examined whether ghrelin has proliferative and or apoptotic effects on these cells. Specific immunostaining for both ghrelin and the GHS-R was observed in all four cell lines. The highest level of intensity of staining for both ligand and receptor was observed in the LDN, with decreasing intensity in the Ishikawa, HEC1A and AN3CA cell line respectively.

Proliferation experiments were conducted in LDN (3) and Ishikawa cells using a crystal violet assay. Human n-octanoylated ghrelin, for 3 days dose-dependently increased proliferation at the optimal dose (10^{-9}M), proliferation was 141+/-6 (LDN) and 137 +/-8 (Ishikawa) of untreated controls, ($P<0.05$ vs control, n=5).

Apoptosis was measured by flow cytometry (propidium iodide staining for DNA content) in the LDN cells following culture for 3 days +/- ghrelin (10^{-9}M). Optimal concentration was determined from a pilot response study. Ghrelin reduced the apoptosis to 30%+/-9 of untreated controls ($P<0.05$, n=3). These results indicate a potential tumour promoting and anti-apoptotic roles for ghrelin in endometrial cancer.

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(2) Tawadros et al, Proc ESA 2002
(3) DiNezza et al, Gynae Oncol 89:325-33

183

DILEMMAS IN THE MANAGEMENT OF PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

N. Arnold [1], M. McLean [1], S. Boyages [1], D. Chipps [1], R. Coles [2], C. Eastman [1], J. Hazel [1], T. Hng [1], J. Holmes-Walker [1], J. Marks [1], H. Smith [1], N. Cheung [1,2]

[1] Centre for Diabetes and Endocrinology Research, Westmead Hospital, Westmead, NSW, Australia
[2] Department of Endocrinology, Nepean Hospital, NSW, Australia

The management of differentiated thyroid carcinoma (DTC) when Radioiodine Whole Body Scans (WBS) and serum. Thyroglobulin levels (Tg) are discordant is not well established We conducted a retrospective review of patients after surgery and Radioactive Iodine (RAI), who had concurrent Tg and WBS in the hypothyroid state, initially and/or on follow up. We examined characteristics of patients with DTC who had discordant WBS and Tg results. These are the preliminary findings of 91 patients admitted between 1990 and 1999. After initial post-operative ablative RAI treatment, Tg and WBS were both positive in 46 patients and both negative in 1 patient. Tg was positive and WBS negative in 1 patient both initially and during follow-up and in 10 further patients during follow up. WBS was positive and Tg was negative in 11 patients initially and remained so in 3 of these patients during follow up. During follow-up, 9 further patients (12 patients in total) were found to have a negative Tg at the time of a positive WBS. Of these 12 patients, 11 were female, 54% had non-malignant thyroid disease and 33% had a family history of thyroid disease. Follicular carcinoma comprised 25% of this population, 50% of papillary carcinomas were multicentric and 70% had benign thyroid disease on histological examination. WBS showed uptake in the thyroid bed and neck only. The 12 patients were in clinical remission at last follow-up (median 9 years, range 4.7-14.8 years). Tg remained negative in all patients and Tg antibodies were present in 50% of patients. We will examine the clinical and pathological correlates of patients with discordant Tg and WBS results that may facilitate better management decisions in the follow up of patients with DTC.

184

HETEROGENEITY OF FAMILIAL PARAGANGLIOMA SYNDROMES IN FOUR PATIENTS WITH SDHB MUTATIONS

D. E. Henley [1], P. T. Pullan [1,2], D. E. Benn [3,4], D. J. Marsh [3,4], B. G. Robinson [3,5]

[1] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
[2] Department of Medicine, University of Western Australia, Nedlands, WA, Australia
[3] Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney, NSW, Australia
[4] Department of Molecular Medicine, University of Sydney, Sydney, NSW, Australia
[5] Department of Medicine, University of Sydney, Sydney, NSW, Australia

Paragangliomas are catecholamine secreting tumours arising from the extra-adrenal sympathetic ganglia. Recently, mutations in the succinate dehydrogenase subunit B and D (SDHB and SDHD) genes have been discovered in association with familial paraganglioma syndromes. We present four patients, each with a different SDHB gene mutation, to illustrate the heterogeneity of this condition. There were 3 males and 1 female of ages 15 to 53 years. Three patients had a family history of paraganglioma and the fourth had a new mutation. One asymptomatic patient had a gene defect

discovered during family screening and apart from a mildly elevated plasma chromogranin A, has no biochemical or radiological abnormalities at present. One patient was diagnosed following marked hypertension (up to 195/125 mmHg) during general anaesthesia for an unrelated condition. The other two patients presented with paroxysmal symptoms of sympathetic hyperactivity, including hypertension in the range of 210-230/100-130 mmHg. The latter three patients all had elevated 24 hour urinary catecholamines at diagnosis: noradrenaline ranged from 3910 to 11752 nmol/L (<600nmol/L) and adrenaline 28 to 130 nmol/L (<120nmol/L). Two of these patients had plasma free metanephrines and chromogranin A measured at diagnosis. Normetanephrine ranged from 3825 to 22670 pmol/L (150-700), metanephrine 197 to 2717 pmol/L (90-400) and chromogranin A 51 to 942 U/L (2-18). Thoracic paragangliomas were confirmed histologically in all three of these patients. One of these was MIBG negative but F^{18} FDG PET positive. The other two patients had MIBG avid tumours. One patient was cured (no clinical or biochemical recurrence 8 years later). One patient had normalisation of plasma and urinary catecholamines and the other is on α and β blockade. These four patients demonstrate clinical, biochemical and radiological heterogeneity associated with familial paragangliomas due to SDHB gene mutation.

186

RESTRICTION OF PLACENTAL AND FETAL GROWTH REDUCES GROWTH HORMONE PULSE FREQUENCY IN NEONATES

K. L. Gatford [1], I. J. Clarke [2], M. J. De Blasio [1], J. S. Robinson [1], J. A. Owens [1]

[1] Department of Obstetrics & Gynaecology, University of Adelaide, Adelaide, SA, Australia
[2] Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Poor prenatal growth is characterised by perturbed postnatal growth and body composition, which may be due in part to altered growth hormone (GH) secretion and action. There is some limited evidence for this in humans and experimental animal models of fetal growth restriction. We have shown previously that restricted fetal growth and slow neonatal growth in sheep are associated with elevated circulating GH in adolescent and adult males, but with decreased circulating GH in adult females (1). To determine the age of onset of these alterations in the GH axis, we have investigated the effect of restriction of placental and fetal growth on circulating GH in the neonatal lamb. Placental growth was surgically restricted (PR) by removal of the majority of endometrial implantation sites prior to pregnancy, and ewes were mated following at least 10 weeks recovery. Control (10 male, 9 female) and PR (8 male, 4 female) lambs were born naturally and indwelling catheters were inserted at 5 days of age. GH was measured by specific radioimmunoassay in plasma collected at 10-minute intervals for 5 h at 10 days of age. PR reduced GH pulse frequency (Control 7.9 ± 0.3 peaks/5h, PR 6.2 ± 0.6 peaks/5h; P=0.023), and increased the interval between GH pulses (Control 41.5 ± 2.1 minutes, PR 58.6 ± 8.7 minutes; P=0.038), but did not alter mean or mean baseline GH concentrations, GH pulse amplitude or integrated GH areas (P>0.3 for all). Furthermore, GH pulse frequency at 10 days of age was positively correlated with measures of size at birth, particularly those reflecting soft tissue growth, such as weight and abdominal circumference. Restriction of placental and fetal growth thus reduces the frequency of GH pulses in the neonatal lamb, suggesting that a deprived fetal environment programs the hypothalamic GH pulse generator early in life.

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187

THE ROLE OF AIRE IN THYMOCYTE DEVELOPMENT IN A MURINE MODEL OF TYPE 1A DIABETES

S. P. FOREHAN [1,2], G. DAVEY [1], J. MCCLUSKEY [2], H. SCOTT [1], W. HEATH [1]

[1] IMMUNOLOGY DIVISION, THE WALTER & ELIZA HALL INSTITUTE, PARKVILLE, VIC, Australia

[2] DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY, THE UNIVERSITY OF MELBOURNE, PARKVILLE, VIC, Australia

RATIONALE: Central tolerance to ubiquitous self-antigens is well recognised. Given that tissue specific antigens are less abundant, peripheral rather than central tolerance mechanisms have been invoked to explain tolerance to such antigens. Recently, however, the autoimmune regulator (AIRE) has been implicated in the development of central tolerance to tissue specific self-antigens, eg insulin. Loss of function mutations in this gene result in Autoimmune Polyglandular Syndrome type I (APS-I) - a syndrome which involves autoimmune targeting of multiple specific tissues. AIRE encodes a putative transcriptional activator that induces promiscuous gene expression in the thymus and facilitates negative selection of developing thymocytes (including those with autoreactive potential). Failure to negatively select autoreactive T cells may result in autoimmune diseases such as type 1A diabetes. METHODS: A knock-out model of the murine homologue, Aire, has been used to assess its effect on the development of endogenous ovalbumin(OVA)-specific transgenic thymocytes in mice that also express OVA in the pancreas as a neo-self antigen. Numbers of OVA-specific T cells in the thymus have been quantitated by flow cytometry. RESULTS: Deletion of OVA-specific thymocytes has been demonstated in aire$^{+/+}$ mice expressing OVA in a tissue specific manner. In aire$^{o/o}$ mice expressing tissue specific OVA fewer specific thymocytes are deleted. CONCLUSIONS: These data support previous observations that Aire facilitates the deletion of auto-reactive thymocytes, a finding that has important implications for tissue specific autoimmune disease.

188

VITAMIN D DEFICIENCY IN PREGNANCY: POSTNATAL AUDIT OF VITAMIN D AND BONE HEALTH IN WOMEN AND THEIR INFANTS.

M. R. Zacharin [1], R. Morley [2], S. Grover [3], K. Thomson [1]

[1] Endocrinology & Diabetes, Royal Children's hospital, Parkville, VIC, Australia

[2] Department of Paediatrics and Murdoch Childrens Research Institute, University of Melbourne, Melbourne, VIC, Australia

[3] Royal Women's Hospital, Maternity Services, Melbourne, VIC, Australia

Objectives: To audit effectiveness of treatment and counselling of vitamin D deficient pregnant women, and prevalence of vitamin D deficiency and bone disease in their infants. A clinical audit at the Royal Children's Hospital, Melbourne was undertaken of 69 women delivering at the Royal Women's Hospital Melbourne who had vitamin D (25-OHD) level <30nmol/L in pregnancy, and their infants at age 4-10 months. Maternal and infant vitamin D, alkaline phosphatase (ALP), parathyroid

hormone (PTH), calcium and phosphorus levels were measured. X-rays of children with clinical or laboratory findings suggestive of rickets were performed Results: 47/69 (68%) mother-infant pairs attended. Among these, 35 women (74%) had been prescribed vitamin D supplements and 19/35 (54%) reported having taken them as prescribed. All 47 women had 25-OHD <50nmol/L and 39 (83%) had levels <30nmol/L. Eighteen of the 45 infants (40%) had 25-OHD <50nmol/L with 14 infants (31%) <30nmol/L. Breast fed infants were more likely to have 25-OHD level below 30nmol/L) than those fed formula (12/16 versus 2/29 respectively, p=0.001 by chi-squared). Two participating infants and a sibling were diagnosed with rickets. Conclusions : Treatment and counselling of pregnant women with vitamin D deficiency are inadequate as most are also vitamin D deficient post-natally. Their infants are at high risk of vitamin D deficiency and should be supplemented if breast fed. Rickets may be far more prevalent than we appreciated in such high-risk populations.

189

PLACENTAL RESTRICTION IMPAIRS GLUCOSE HOMEOSTASIS IN ADULT RATS, INDEPENDENTLY OF ADIPOSITY AND CIRCULATING FREE FATTY ACIDS

F. M. Leone [1], M. Wlodek [3], A. L. Mibus [3], O. Wyss [2], D. Horton [2], K. T. Westcott [3], M. Walker [1], V. Staikopolous [3], J. S. Robsinson [1], J. A. Owens [1]

[1] Obstetrics & Gynaecology, University of Adelaide, Adelaide, SA, Australia
[2] Physiology, University of Adelaide, Adelaide, SA, Australia
[3] Physiology, University of Melbourne, Melbourne, VIC, Australia

Intrauterine growth restriction (IUGR) is associated with an increased prevalence of insulin resistance (IR) and non-insulin dependent diabetes mellitus (NIDDM) in adult humans. The main cause of IUGR in the western society is placental insufficiency, but whether this causes NIDDM in humans is unknown, while different studies of experimental placental restriction in the rat have produced conflicting outcomes for glucose homeostasis. We therefore determined the effect of placental insufficiency on glucose tolerance in adult male and female rat offspring. As IUGR and catch-up growth also are associated with adult obesity, we further hypothesised that placental insufficiency causes NIDDM in part by increasing adiposity and free fatty acids (FFA). Bilateral uterine vessels were ligated on day 18 of gestation (BUVL) and offspring studied at 26 weeks of age. Sham-operated pregnant rats with normal litter size (Sham) and with a reduced litter size (Sham Reduced) served as controls. BUVL reduced neonatal and adult weight when compared to Sham ($p< 0.05$) and Sham Reduced ($p<0.05$). BUVL increased fasting blood glucose ($p<0.05$) in adult offspring, but impaired glucose tolerance ($p<0.05$) in males only compared to Sham. BUVL also increased adiposity in female offspring ($p<0.05$), but did not alter plasma FFA in either sex. Therefore, placental restriction reduces neonatal and adult size and impairs glucose homeostasis in adult offspring in both sexes, independently of adiposity and circulating FFA.

THYROID HORMONE METABOLISM AND BIRTH WEIGHT

T. M. Hng , N. W. Cheung , M. McLean

Centre for Diab & Endocrinology Research, Westmead Hospital, Westmead, NSW, Australia

INTRODUCTION AND OBJECTIVE Birth weight (BW), a surrogate marker for intra-uterine stress, is associated with differences in body composition. As thyroid hormones play an active role in tissue metabolism, we sought to determine if changes in thyroid hormone metabolism might contribute to the effects on body composition arising from low BW. T4 is metabolised to T3 or the inactive rT3 through deiodinases. Changes in deiodinase activity, resulting in an alteration in T4 metabolism, can result in chronic changes in tissues due to subtle over or under activity. The relative activity of the deiodinases can be deduced by examining the ratio of T3 to rT3. A twin model was utilised to minimise the effect of genetic confounders. DESIGN AND METHODS 19 sets of adult same sex twins were recruited via the Australian Twin Registry. Birth records were obtained. Fasting blood samples were obtained from each twin set on the same day. TSH, fT4, fT3 and rT3 were assayed. Results were analysed by paired t-test and descriptive data are stated as mean ± SD. RESULTS The mean age was 39.3 ± 10.4 years. There were 17 female and 2 male twin sets. 13 sets were monozygotic (1 male). Mean BW for all individuals was 2496.6 ± 503.9 grams and intra-pair BW difference was 311 ± 210.4 grams. There were no significant intra-pair differences in plasma TSH or fT4 levels. Intra-pair examination of the fT3 to rT3 ratio for the monozygotic, dizygotic and total groups respectively did not reveal any significant. There was no correlation of differences in birth weight and differences in the ratio of ft3 to rT3. CONCLUSION In twin pairs, the activity of the enzymes metabolising T4 does not appear to be influenced by weight at birth. This suggests that alterations in adult body composition are unlikely to be explained by differences in the activity of T4 and T3.

PRENATAL GLUCOCORTICOID EXPOSURE ALTERS LONG-TERM HEPATIC PROTEIN EXPRESSION IN SHEEP

D. M. Sloboda [1], T. J.M. Moss [1], S. Li [1], J. R.G. Challis [2], J. P. Newnham [1]

[1] School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia

[2] Physiology and Obstetrics and Gynecology, The University of Toronto, Toronto, Ontario, Canada

Background: Fetal exposure to glucocorticoids affects development and results in postnatal disease. Clinically, women receive synthetic glucocorticoids to enhance fetal maturation prior to preterm delivery. We have shown that fetal exposure to clinically relevant doses of betamethasone in the sheep results in increased fetal hepatic 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) and corticosteroid binding globulin (CBG) protein levels, as well as changes in postnatal metabolic and stress axis regulation. It is unknown whether changes in hepatic protein expression persist postnatally to potentially alter long-term glucose regulation. *Methods*: Pregnant ewes or their fetuses received either repeated intramuscular saline (MS or FS) or betamethasone injections (0.5mg/kg; M4 or F4) at 104, 111, 118 and 124 days of gestation (dG), or a single betamethasone injection at 104, followed by saline at 111, 118 and 124 dG (term 150 days; M1 or F1). Offspring were killed at 3.5 years of age and liver tissue collected and processed for Western blot and enzymatic analysis. *Results*: Hepatic CBG protein levels were lower in F4 but not F1 animals compared with FS. Glucocorticoid receptor (GR) levels were increased in F1 and F4 animals. Conversely, repeated maternal betamethasone administration resulted in decreased hepatic GR levels. Fetal and maternal treatments resulted in dose dependant increases in glucose-6-phosphatase activity.

GROUP	CBG (AOD)	11βHSD1 (AOD)	GR (AOD)	Glu-6-phosphatase Activity (U/g)
MS	1.069±0.0060	0.129±0.0079	0.90±0.0026	1.246±0.0056
M1	1.055±0.0028	0.130±0.0046	0.89±0.0023	3.735±0.103*
M4	1.040±0.0016	0.116±0.0039	0.88±0.0056*	6.210±0.270*†
FS	1.261±0.0064	1.277±0.0004	1.07±0.0054	0.983±0.021
F1	1.245±0.0010	1.274±0.0015	1.14±0.0083*	5.22±0.075*
F4	1.247±0.0034*	1.272±0.0012	1.16±0.0046*†	7.892±0.166*†

*different from MS or FS; †different from M1 or F1; AOD, arbitrary optical density

Conclusions: Prenatal betamethasone exposure results in dose dependant changes in hepatic protein expression and activity that persist to adulthood. Exposure to prenatal glucocorticoids has severe implications for long-term intrahepatic glucocorticoid regulation and may contribute to hepatic insulin resistance.

192

PLACENTAL RESTRICTION AND SMALL SIZE AT BIRTH ACCELERATE THE INCREASE IN PLASMA LEPTIN WITH AGE IN THE SHEEP

J. A. Owens [1], K. L. Gatford [1], D. Blache [2], M. J. De Blasio [1], J. S. Robinson [1]

[1] Department of Obstetrics & Gynaecology, University of Adelaide, Adelaide, SA, Australia
[2] Faculty of Agriculture, University of Western Australia, Perth, WA, Australia

Leptin is a cytokine, primarily synthesized and secreted by adipocytes, which acts centrally to regulate appetite and energy expenditure and peripherally to influence insulin sensitivity and secretion. Restriction of growth before birth is implicated as permanently changing these various determinants of metabolic homeostasis in postnatal life, but the role of leptin in this is poorly understood. Adults who were small at birth have increased plasma leptin, which may reflect increased adiposity. Low plasma leptin or leptin resistance may precede this however, as rats, guinea pigs and sheep that underwent fetal growth restriction have increased appetite in early postnatal life or as adults. We therefore hypothesised that placental restriction of fetal growth and size at birth in sheep would reduce plasma leptin in juveniles, but increase plasma leptin in adults. Offspring of control sheep and sheep in which placental growth was surgically restricted by removal of the majority of endometrial implantation sites prior to pregnancy, were studied as lambs through to early adulthood: 21 control (13 male, 8 female) and 25 placentally-restricted (9 male, 16 female) lambs at 130 (juvenile), 375 (young adult) and 550 (adult) days. Vascular catheters were inserted under general anaesthesia and asepsis at least 7 days prior to blood sampling. Plasma leptin was measured by an ovine specific radioimmunoassay. Effect of treatment, sex and age were examined by ANOVA. Body composition was determined at post-mortem at 550 days of age. Plasma leptin was higher in females than males ($p=0.001$), increased with age ($p<0.0001$) and to a greater extent in placentally restricted compared to control sheep between 130 and 550 days ($p=0.035$), and was higher in placentally restricted compared to control sheep at 550 days of age (2.05 ± 0.16 ng/ml vs 1.56 ± 0.15 ng/ml) ($p<0.05$). Plasma leptin in adult sheep correlated positively with perirenal fat (absolute and relative to body weight) in males ($r=0.439$, $p=0.03$; $r=0.33$, $p=0.09$) and females ($r=0.611$, $p=0.005$; $r=0.548$, $p=0.011$), due mainly to associations following placental restriction. Therefore placental restriction of fetal growth and size at birth accelerated the age related increase in plasma leptin, which may result from more rapidly increasing adiposity.

SEX DIFFERENCES IN IMPAIRED GLUCOSE HOMEOSTASIS IN THE YOUNG ADULT GUINEA PIG WITH SPONTANEOUS FETAL GROWTH RESTRICTION.

S. Grover [1,2], C. L. Coulter [2], M. R. Walker [1], K. L. Kind [1], J. S. Robinson [1], J. A. Owens [1]

[1] Department of Obstetrics and and Gynaecology, The University of Adelaide, Adelaide, SA, Australia

[2] School of Molecular and Biomedical Science, Discipline of Physiology, The University of Adelaide, Adelaide, SA, Australia

Spontaneous fetal growth restriction (FGR) in the guinea pig is associated with insulin resistance (IR) in the young adult, as in humans, but whether this impairs glucose homeostasis is unclear. We therefore determined the effect of spontaneous FGR on fasting blood glucose (FBG), maximum blood glucose (MBG) and glucose tolerance (GT) in the young adult guinea pig. Size at birth was measured in terms of weight (BWT), crown rump length (CRL), abdominal circumference (AC), head width and length. Animals were divided into tertiles according to BWT, low >90g, medium 90-110g and high <110g. Vascular catheters were implanted at 100 ± 3 days of age and following a five-day recovery period, an intravenous glucose tolerance test (IVGTT) was performed (0.5g/kg at 0 min; blood was sampled frequently between –10 and 210 min). FBG was determined as the mean of three samples obtained prior to glucose bolus, MBG was determined at 2 min directly after the infusion of the glucose bolus and GT was determined by calculating the glucose area under the curve (GAUC). GT varied with birth weight tertile ($p<0.06$). Offspring of low BWT had impaired GT compared to medium and high BWT ($p=0.039$, 0.026 respectively). When genders were separated BWT tertile did not influence GT in males. However in females GT varied with BWT tertile ($p=0.009$), with the low BWT group having higher GAUC than medium and high BWT groups ($p=0.003$, $p=0.014$ respectively). Correlation analysis in males showed that, FBG, MBG ($p<0.05$) and GAUC tended to ($p<0.1$) correlate negatively with CRL, AC and head size at birth. In females, GAUC correlated negatively with size at birth in terms of all parameters measured ($p<0.05$). Spontaneous FGR is therefore associated with impaired GT in both male and female young adult guinea pigs and with increased fasting blood glucose in males only. This provides evidence that adverse consequences of early life programming are of earlier onset and greater magnitude in males compared to females. This is consistent with our previous observations that spontaneous FGR causes hepatic as well as skeletal muscle IR in young adult males, but only the latter in young adult females.

POSTERS

302

DISTRIBUTION AND CO-EXPRESSION OF ENKEPHALIN AND PEPTIDES OF THE STRESS AXIS IN THE PARAVENTRICULAR NUCLEUS OF MALE AND FEMALE SHEEP

E. T.A. Rivalland [1], J. Iqbal [2], A. I. Turner [1], I. J. Clarke [2], A. J. Tilbrook [1]

[1] Physiology, Monash University, Monash University, VIC, Australia

[2] PHIMR, Clayton, VIC, Australia

The paraventricular nucleus (PVN) is central to activation of the hypothalamo-pituitary-adrenal (HPA) axis. Within this nucleus, cells produce corticotrophin-releasing hormone (CRH), arginine vasopressin (AVP) and enkephalin (ENK); CRH and AVP are secreted into the hypophysial portal system in response to various stressors, but this is influenced by sex and sex steroids. In order to study this system in the ovine, it is first necessary to map the distribution of the relevant peptides and the extent to which they co-localise within cells of the PVN. Thus, our aim was to define the distribution of immunoreactive (ir) CRH, AVP and ENK producing cells in the PVN of male and female sheep and to determine the extent of co-localisation of these peptides in intact and gonadectomised animals. Brains from gonadectomised and intact Corriedale sheep (n=4/group) were perfusion fixed for immunohistochemistry. Hypothalami were sectioned (30 µm) and immunohistochemistry was performed for CRH, AVP and ENK-ir at 180µm intervals throughout the rostro-caudal extent of the PVN. Maps for each peptide were generated in two rams and two ewes. The distribution of AVP-ir cells was similar in both sexes but CRF and ENK-ir cells were observed more rostrally in rams. Double-immunofluorescence was carried out in all animals for CRH/AVP, CRH/ENK and AVP/ENK, to determine the extent of co-localisation. The percentage co-localization of neuropeptides at different levels of the PVN was influenced by gonadectomy. In rostral and central regions of the PVN, the number of AVP cells that also stained for ENK was greater ($P<0.05$) in intact animals than in castrates (sexes combined). Gonadectomised animals, however, had a greater ($P<0.05$) percentage of AVP cells containing ENK and ENK cells containing AVP in the caudal PVN. No other sex differences were seen. The difference in co-localization may provide an anatomical basis for the sex differences observed during stress.

303

IDENTIFICATION OF A DNA BINDING PROTEIN FAMILY WITH SIMILARITY TO RNA SPLICING FACTORS

K. L. Shipman [1], J. Stewart [1], P. J. Robinson [2], R. Smith [1], R. C. Nicholson [1]

[1] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

[2] Children's Medical Research Institute, Sydney, NSW, Australia

The cAMP regulatory element (CRE) is a crucial regulatory element controlling corticotropin releasing hormone (CRH) expression in both the hypothalamus and placenta. Our previous studies have focused on the mechanisms of placental specific CRH production with the yeast-one hybrid system being used to functionally screen a placenta cDNA library to identify placental nuclear

proteins that bind the CRE. A cDNA clone was identified that was not homologous with any known transcription factor and encoded a protein that was found to specifically bind the CRE using EMSA. This protein has been named CREAP, for CRE Associated Protein. CREAP is particularly highly expressed in fetal lung, thymus, kidney and brain and in adult cerebellum, pituitary and thymus. Comparison of the predicted human CREAP amino acid sequence with the protein databases indicates that there are two highly related proteins that share the same structural features as CREAP. These features include two leucine zipper-like domains with DNA binding potential, two coiled-coil domains with protein-protein interaction potential, DNA and RNA binding-type zinc fingers and a SR rich domain similar to those found in RNA-splicing proteins. No function is yet known for the proteins identified in the database but our analysis predicts that this new family of proteins has the potential to interact with DNA and RNA, as well as proteins. The CREAP mRNA is expressed as a variety of spliced variants. These variants encode long and short versions of the protein, with and without the SR-rich domain. Analysis of proteins in placental and other fetal tissues using CREAP anti-sera and Western blotting indicates that these protein variations are observed at a cellular level. The possibility that CREAP may be a self-splicing protein is now under investigation.

304

DESPITE DIFFERENCES IN BODY COMPOSITION IGF-I LEVELS ARE SIMILAR IN SAMOAN, MAORI AND EUROPEAN POPULATIONS.

W. Bagg [1,2], J. Aoina [1], P. A.R. Cross [1], G. A. Whalley [1], G. D. Gamble [1], R. N. Doughty [1], I. M. Holdaway [2]

[1] Medicine, University of Auckland, Auckland, New Zealand
[2] Endocrinology, Auckland City Hospital, Auckland, New Zealand

The reference range of serum IGF-I concentrations in people of Samoan and Maori descent have been assumed to be similar to the European reference range. However, recent studies have suggested that ethnic differences may contribute to variance in IGF-I concentrations. Aim: To measure serum IGF-I concentrations in Samoan, Maori and European adults and determine if there is any relationship between IGF-I and body composition that may explain any differences. Methods: Healthy adults had body composition assessed by DEXA and LVM by echocardiography. IGF-I measured by Immulite 2000 immunoassay. Results:

	European n=30)	Maori (n = 22)	Samoan (n = 23)	ANOVA
Mean age (yrs)	28 ± 6	28 ± 6	31 ± 7	0.80
% male	44	46	53	0.81
Weight (kg)	76.2 ± 19.7	81.9 ± 20	94.5 ± 20.8	0.006
BMI (kg/m^2)	24.8 ± 4.9	28.0 ± 6.2	31.8 ± 7.0	0.0003
Fat free mass (FFM) (kg)	51.0 ± 13.0	57.3 ± 148.8	59.8 ± 13.6*	0.06
Fat mass (kg)	21.5 ± 9.7	23.8 ± 12.4	30.1 ± 13.3$^{\#}$	0.32
IGF-I (ug/l)	186.8 ± 87.4	204.8 ± 63.9	180.0 ± 85.5	0.58

Samoan vs European *FFM and $^{\#}$fat mass p = 0.009 and p = 0.017.

There was a strong inverse relationship between IGF-I and age (r = -0.50, p < 0.0001) but there was no relationship between IGF-I and sex or ethnicity (both p > 0.50). IGF-I was inversely related to fat mass and percentage fat (r = -0.25, p =0.04; r = -0.23, p = 0.04) but not to FFM (r = -0.05, p = 0.66). Adjustment for age and fat mass did not produce a difference in IGF-I between ethnic groups (p = 0.44). Conclusions: Despite significant differences in body composition, especially between the

Samoan and European groups, IGF-I concentrations were not different suggesting that use of current reference ranges is appropriate for these ethnic groups.

305

THE FIRST INTERNATIONAL RANDOMIZED TRIAL IN LOCALLY ADVANCED AND METASTATIC ADRENOCORTICAL CARCINOMA TREATMENT (FIRM-ACT)

D. J. Torpy [1], I. N. Olver [2]

[1] Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA, Australia
[2] Cancer Centre, Royal Adelaide Hospital, Adelaide, SA, Australia

Adrenocortical carcinoma is rare (1-2 per million per year). Five-year survival of adrenocortical carcinoma is 23-60% and the median survival of patients with Stage IV disease is only 25 weeks. Currently, therapy often includes mitotane (o.p'-DDD) and single or multi-agent chemotherapy. There are few studies of more than 10 patients; hence the optimum therapy is unknown. The rarity of the disease limits the potential for study sponsorship. The 2003 International Consensus Conference on Adrenal Cancer recommended that the best two options, based on current evidence, are etoposide, doxorubicin, cisplatin and mitotane (EDP+Mitotane) or streptozotocin and mitotane (S+Mitotane). Both regimens used low-dose (1-4 gm/day) mitotane. This recommendation was based on recently reported response rates of 54% in 28 patients to EDP+Mitotane[1], and 36% in 22 patients to S+Mitotane.[2] The FIRM-ACT study will recruit 300 patients with locally advanced or metastatic (Stage III-IV) adrenocortical carcinoma that is not amenable to radical surgical excision. Patients will be randomized to one of the two recommended treatment protocols (EDP+Mitotane or S+Mitotane) with survival as the primary end-point. Patients exhibiting a poor treatment response on 8-12 weekly follow-up, or toxicity, will be allocated to the alternative as a second line. Blood mitotane levels will be monitored, as response appears to correlate with mitotane levels of 14-20 mg/L. Involvement of oncologists and endocrinologists in major Australian cities could collaboratively make an important contribution to this international study over its planned seven year duration.

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(2) 2. Khan TS, Imam H, Juhlin C, et al. Ann Oncol 2000;11(10):1281-1287.

306

AROMATASE IS NOT EXPRESSED IN ENDOMETRIAL CANCER

N. A. Pathirage [1,2], C. D. Clyne [1,2], E. R. Simpson [1,2]

[1] Phimr, Monash University, Clayton, Vic, Australia
[2] Department Of Biochemistry And Molecular Biology, Monash University, Clayton, Vic, Australia

Introduction: Aromatase expression and *in situ* estrogen production have been described in endometrial cancer stroma (but not in normal endometrium), where locally-produced estrogens have been postulated to influence tumor growth and progression *via* paracrine and autocrine mechanisms. Conflicting data exists regarding the levels of aromatase expressed in endometrial cancer. Thus, to assess the potential of these tumors to synthesise and respond to estrogens, this study quantified mRNA expression of aromatase, estrogen receptor (ER) alpha, ER beta and the ER co-activator SRC-1 in a panel of normal (n=20) and cancerous (n=16, histological grade 1-3) endometrial biopsies.

Methods: Total RNA was isolated from endometrial tissues or from cultured human adipose stromal cells (as a positive control), then reverse transcribed and transcripts quantified by real-time PCR using internal standards of known concentration. *Results*: Aromatase was not detected in both the normal and the cancerous endometrium, but was readily detectable in the positive control (mean levels 123.52 +/-23 fg/ugRNA). Histologic Grade 1 tumors (highly differentiated) showed a significant increase in ER-alpha, ER-beta and SRC-1 expression compared with Grade 2 and Grade 3 tumors ($P<0.05$ and $P<0.05$, respectively). *Conclusions*: There was no evidence of aromatase expression in any of the endometrial samples examined. However, ERs and SRC-1 were detected in all samples and showed a negative correlation with tumour grade, consistent with the ability of the tumors to respond mitogenically to estrogens, and with the inability of less differentiated tumours to respond to hormones. Further work is required to elucidate the role of estrogen signalling in endometrial cancer.

307

NOVOBIOCIN INHIBITS CROSS-TALK BETWEEN HEAT SHOCK PROTEIN 90 DIMERISATION AND ATP-BINDING DOMAINS

R. K. Allan [1,2], B. K. Ward [1,2], D. Mok [1,2], R. L. Matts [3], T. Ratajczak [1,2]

[1] Western Australian Institute for Medical Research and the UWA Centre for Medical, The University of Western Australia, Perth, WA, Australia

[2] Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Perth, WA, Australia

[3] Biochemistry & Molecular Biology, Oklahoma State University, Oklahoma, Oklahoma State, United States

Heat shock protein 90 (Hsp90) has an essential role in the activation of a number of key regulatory and signalling proteins including steroid receptors (SRs) and protein kinases. Client protein activation requires Hsp90 ATPase function and can be disrupted by geldanamycin, a competitive inhibitor of the ATP binding site located in the Hsp90 N-terminal domain. Hsp90 forms distinct heterocomplexes with SRs and protein kinases by associating with tetratricopeptide repeat (TPR)-containing immunophilins or $p50^{Cdc37}$, respectively, and these cochaperones directly interact with the client proteins to provide additional selection specificity. $p50^{Cdc37}$ recruits kinases by interacting with the Hsp90 N-terminal domain to block Hsp90 ATPase function, thus facilitating client kinase loading (1). The TPR-immunophilins on the other hand compete for a common TPR acceptor site in the Hsp90 C-terminal domain, a region important for Hsp90 dimerisation and SR interaction. By enhancing Hsp90 ATPase function, the immunophilins may promote more efficient SR activation. Hsp90 is a target for novobiocin, a coumarin-related DNA gyrase inhibitor which binds to the Hsp90 C-terminal dimerisation domain and destabilises several proto-oncogenic kinases *in vivo* (2). Our laboratory has previously shown that novobiocin can differentially disrupt Hsp90 binding to TPR-immunophilins. In the present study we have used a microtitre plate assay for Hsp90- $p50^{Cdc37}$ binding to demonstrate that the drug can inhibit this interaction *in vitro*. Our results suggest that novobiocin can interfere with functional interactions between the C-terminal and N-terminal domains of Hsp90. We are currently investigating the effects of novobiocin on glucocorticoid receptor function in HeLa cells by assessing luciferase reporter activity.

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(2) Roe et al (2004), Cell, 116, 87-98.

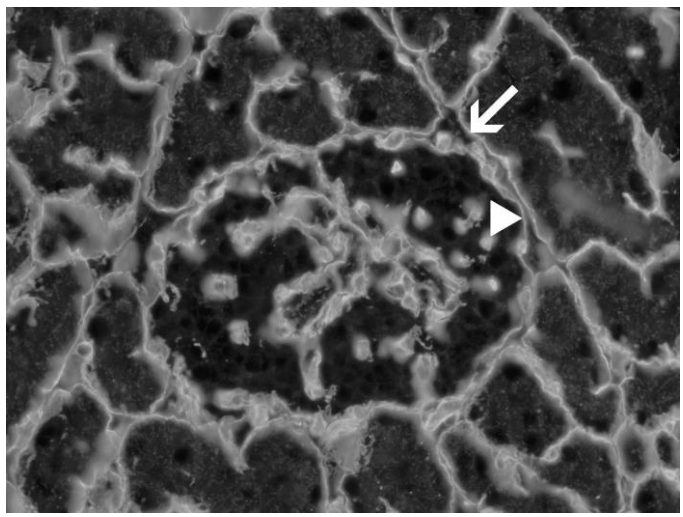
COMPOSITION OF THE MURINE PANCREATIC ISLET BASAL LAMINA

H. F. Irving-Rodgers [1], R. J. Rodgers [1], C. R. Parish [2], A. Ziolkowski [2], C. J. Simeonovic [2]

[1] Obstetrics and Gynaecology, University of Adelaide, Adelaide, SA, Australia

[2] The John Curtin School of Medicine, The Australian National University, Canberra, ACT, Australia

Basal laminas are complex extracellular matrixes composed of collagen type IV, laminins, nidogen and heparan sulphate proteogylcans. Some of these components also localise to the extracellular matrix but lack basal lamina morphology. Basal laminas function as selective barriers, provide physical support to cells, and their individual components have biological activities influencing cellular functions such as growth, differentiation and migration. Conflicting information exists as to the actual presence of a basal lamina surrounding the pancreatic islet (Jiang et al., 2002, van Deijnen et al., 1992). We have immunolocalised specific individual basal lamina components [Collagen type IV (a2), laminin chains (a2, b2), nidogen (1, 2), and the heperan sulphate proteoglycan perlecan] to Non-Obese Diabetic (NOD) mouse pancreas. A layer of material immuno-positive for the above surrounds the pancreatic islet (arrowhead) and acini (arrow). By electron microscopy there is a layer of electron dense material surrounding the pancreatic islet. Therefore we would conclude that the pancreatic islet is surrounded by a basal lamina. The role of this basal lamina in the development of autoimmune diabetes is under investigation.



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309

ACTH-INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA: A RARE CAUSE OF CUSHING'S SYNDROME

M. Grossmann [1], R. Wong [1], F. Long [1], W. R. Johnson [2], D. J. Topliss [1]

[1] Ewen Downie Metabolic Unit, Alfred Hospital, Melbourne, VIC, Australia
[2] Breast and Endocrine Surgical Unit, Alfred Hospital, Melbourne, VIC, Australia

A 50 yo woman presented with a two-year history of weight gain (10 kg), easy bruising, sleep apnoea and mental vagueness. There was no significant past or family medical history. Examination findings were truncal obesity, moon facies, atrophic skin, proximal myopathy, and blood pressure 170/100. DEXA BMD values were in the osteopaenic range. Urinary free cortisol was 2323 (repeat 2025) nmol/24h, and 0800h cortisol 599 nmol/L after 1 mg dexamethasone at 2400h. Plasma ACTH values were all < 1 pmol/L and 4 mg dexamethasone infusion failed to suppress cortisol (all > 480 nmol/L), consistent with a primary adrenal source. Abdominal CT showed bilateral adrenal nodules up to 2.5 cm. Pituitary MRI was normal. Adrenal vein sampling demonstrated bilateral cortisol secretion. She underwent uneventful bilateral laparoscopic adrenalectomies and was commenced on replacement therapy with cortisone acetate, fludrocortisone and DHEA. Combined adrenal weight was 55 g (normal < 12 g) and histology revealed multiple adrenal nodules with internodular cortical atrophy. ACTH-independent macronodular hyperplasia is an unusual cause of Cushing´s syndrome, with about 50 cases reported in the literature with a combined adrenal weight range of 17-300g (1). It may be caused by aberrant adrenal hormone receptor expression (2). Bilateral total adrenalectomy is usually curative, and Nelson´s syndrome has not been observed.

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(2) Lieberman SA et al., Eur J Endocrinol 131:67, 1994

310

AUSTRALIAN AND NEW ZEALAND ACROMEGALY REGISTRY (ANZAR): PROGRESS IN DEVELOPMENT OF A COLLABORATIVE RESOURCE

M. McLean [1], R. Cuneo [2], K. Ho [3], W. Inder [4], I. Holdaway [5], S. Judd [6], L. Atkinson [7]

[1] Diabetes and Endocrinology, Westmead Hospital, Westmead, NSW, Australia
[2] Metabolic Research Unit, Princess Alexandra Hospital and University of Queensland, Brisbane, QLD, Australia
[3] Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia
[4] Endocrinology, St Vincent's Hospital, Melbourne, VIC, Australia
[5] Endocrinology, Auckland City Hospital, Auckland, New Zealand
[6] Medicine, Flinders Medical Centre, Adelaide, SA, Australia
[7] Neurosurgery, Princess Alexandra Hospital, Brisbane, QLD, Australia

ANZAR is a collaborative venture open to all clinicians involved in management of patients with acromegaly. It aims to collate data relating to the prevalence, demography, treatment and clinical outcome of this rare disorder. The registry has been endorsed by the ESA and has received seed funding from pharmaceutical industry sponsors. Additional government funding is also being sought. The project will be a multi-disciplinary collaboration of clinicians from endocrinology, neurosurgery, radiation oncology and related fields. A Steering Committee has been formed and the clinical data set has been defined. Various models of computerised database have been considered and trialed at

individual institutions. A paper-based method of data capture has been developed, with electronic reporting likely to follow in time. Wide consultation has been undertaked regarding the ethical issues of centralised disease registries. All data will be de-identified before being submitted. Six institutions have so far applied for or obtained approval from local ethics committees; and data from >500 patients will be available. It is hoped that further institutions and clinicians will join the collaboration. Data reported to date indicates considerable variation in management between centres. Overall, 85% of patients were treated surgically, 64% received radiotherapy, 15% had primary medical therapy. Analysis of patient outcomes by treatment category will be presented. ANZAR will be used to identify the incidence/prevalence of acromegaly in our region; past and current trends in management; and to assess the effectiveness, cost and adverse effects of different treatment modalities. Analysis of pooled patient data is particularly important in rare disorders where controlled trial data is difficult to obtain, and where no single centre is able to gain extensive local experience. All clinicians involved in the care of patients with acromegaly are invited to participate in this on-going project.

311

CUSHING'S SYNDROME AS A MANIFESTATION OF CARNEY COMPLEX: A CASE REPORT

K. Milner , M. Burt , K. K.Y. Ho

Department of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia

A 22 year old female presented with a several year history of 10kg weight gain, secondary amenorrhoea, easy bruising, headaches, decreased muscle strength, depression, hypertension and recurrent urinary tract infections. Examination revealed truncal adiposity with thin extremities, moon facies, buffalo hump, purple striae on legs, atrophied skin with no nodules or pigmentation and proximal myopathy. There were no visual field or ocular defects. 24hr urinary free cortisol excretion was elevated ranging from 600 to 2000nmol/day and was unaffected by fasting. Plasma cortisol failed to suppress after a 1mg overnight dexamethasone suppression test (733nmol/L) or to a 5hr intravenous dexamethasone infusion (baseline 516nmol/L, 5hr 527nmol/L) and ACTH was undetectable throughout. Baseline pituitary function tests were normal. The pituitary gland was normal on MRI. Both adrenal glands appeared slightly enlarged on CT scan with no focal nodules. An ^{131}I-methylnorcholesterol scan demonstrated equal uptake in both adrenal glands. Ketoconazole was commenced to achieve biochemical control in preparation for bilateral laparoscopic adrenalectomy. Microscopic examination of both adrenals revealed multiple pigmented hyperplastic nodules. These features are typical of the Carney complex which is a multiple neoplasia syndrome of endocrine (thyroid, pituitary, adrenocortical and gonadal), non-endocrine (myxomas, naevi and cutaneous pigmented lesions) and neural (schwannomas) tumours. It is inherited as an autosomal dominant trait and a predominant chromosomal locus has been identified at 17q22-24. A mutation of the protein kinase A gene has been identified in over 50% of cases. This patient is being investigated for other clinical manifestations of this complex. Other family members have been noted with cushingoid features. Carney complex is a rare cause of ACTH independent Cushing's syndrome. Its genetic basis calls for screening of family members, which is currently being undertaken..

312

CHALLENGES IN THE MANAGEMENT OF ADRENOCORTICOTROPIN RELEASING HORMONE[ACTH] SECRETING PITUITARY MACROADENOMAS.

I. A.J. Jayasuriya , P. J. Fuller

department of endocrinology, monash medical centre, clayton, VIC, Australia

We report two cases of ACTH - secreting pituitary macroadenoma with an unusual presentation with the typical manifestations and without the usual manifestations of Cushing's disease. A 46 year old man presented in August 2003 with a febrile illness, seizures, confusion and newly diagnosed hyperglycemia [glucose 55mmol/l]. His other background medical problems included a significant bipolar disorder which was treated with lithium. He had been complaining of polyuria and polydipsia for the preceding six weeks which had been attributed to diabetes insipidus. A brain CT scan showed a large 5cm supracellar mass consistent with pituitary macroadenoma. Endocrine evaluation confirmed evidence of panhypopituitarism with a raised ACTH level [45pmmol/l] and random serum cortisol level of 1155nmmol/l. The tumour was resected through a bifrontal craniotomy and histology showed a weakly ACTH staining tumour. He still has some residual tumor activity with normal serum cortisol values. The second case is a 51 years old man who presented with a sudden onset of a right sided third nerve palsy against a background of difficult to control hypertension. A brain CT scan revealed a large suprasellar lesion extending to the cavernous sinus consistent with a pituitary macroadenoma. Subsequent endocrine investigations confirmed increased ACTH [22pmol/l] and nonsupressed cortisol levels[248nmol/l] on 16mg of dexamethasone for three days prior to the serum analysis. The rest of his pituitary investigations were consistent with panhypopituitarism. Transsphenoidal surgery was carried out and histology a showed weak ACTH staining of the tumour. He still has residual tumour mass with significant cortisol production. ACTH secreting pituitary macro adenomas are very uncommon. They do not present with the typical features of Cushing's disease. They are often resistant to the conventional therapy. The management of corticosteroid replacement with the presence of ongoing tumoural ACTH secretion is an added difficulty as is the correct time of pituitary radiotherapy.

313

A CASE OF METFORMIN-INDUCED HYPOGLYCEMIA

A. Omari [2], D. K. Yue [1,2], S. M. Twigg [1,2]

[1] Medicine, University of Sydney, Sydney, NSW, Australia

[2] Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

We report a case of metformin-induced hypoglycemia in the absence of insulin or sulphonylurea therapy. A 57 yr old female with T2DM of 2yr duration had documented macrovascular disease with myocardial infarct 2 yr prior, but not microvascular disease. Intercurrent medications were ramipril 10mg nocte, metoprolol 25 mg bd, indapamide SR 1.5 mg mane, omeprazole 40 mg od, pravastatin 40 mg nocte, and aspirin 100 mg od. Her weight was 72.5 kg with BMI 28.3 kg/m2, BP125/70 mmHg and no cardiovascular abnormalities to examination. Liver function tests were normal, and the serum creatinine 0.09mmol/L, with CrCl 67.4ml/min and microalbumin excretion rate 15.4µg/min. Due to elevated fasting and post-prandial BGLs and HbA1c of 7.6%, metformin was commenced at 425 mg

bd for 1 wk, increasing to 850 mg bd. This was well tolerated and was increased to 850 mg tds aiming for an HbA1c of 6.5%. After 3 months, she had lost 2.5 kg body wt, with home fasting capillary readings of 5.0-6.2 mmol/L, to 8.4 mmol/L post-prandially. She commenced a supervised cardiovascular exercise program and began to develop symptoms of hypoglycaemia less then 30 min after commencing exercise, characterised by sweating, tremor and confusion. Capillary glucose levels at the time of symptoms were from 2.8-3.5 mmol/L. Seven episodes were documented. She could self-treat symptoms with oral carbohydrate, and prevent most but not all episodes by consuming carbohydrate pre-exercise. Morning serum cortisol and thyroid function were normal. The metformin dose was then decreased to 425 mg bd and other medications remained unchanged. Subsequently, despite increased physical activity, hypoglycaemia did not recur. Hypoglycaemia induced by metformin has been reported, including severe episodes and identified risk factors for its occurrence are higher dosing, intercurrent ACEI and NSAID use, and exercise. Whilst it is uncommon, to manage it effectively health professionals and patients need to be aware of this condition.

314

THE MYSTERY OF THE PARATHYROID: A CASE REPORT OF A TWIN PREGNANCY WITH PARADOXICAL MATERNAL AND INFANT OUTCOMES

I. Koves , C. McDonnell , M. Zacharin

Endocrinology & Diabetes, Royal Children's Hospital, Melbourne, VIC, Australia

A female infant (twin 1) presented at 5 weeks of age following a pre-mature dizygotic twin birth at 35 weeks gestation to a covered dark skinned mother. She had a history of irritability and escalating seizure activity over the week preceding admission. Profound hypocalcaemia with ionised calcium nmol/l, low parathyroid hormone <0.1pmol/L and low vitamin D <10nmol/L was found. Twin 2 had a normal calcium of 2.31mmol/L normal PTH and normal vitamin D of 45nmol/L. The mother's serum calcium was 2.76mmol/L, PTH 46.7pmol/L and vitamin D <10nmol/L. Twin 1 required large amounts of calcium and vitamin D supplementation over 3 weeks, with multiple calcium gluconate infusions, 1.5g oral calcium per day, together with a stoss dose of 40,000 units 25 OH vitamin D orally and 1.0mcg/day of 1,25 dihydroxy vitamin D (calcitriol). Serum calcium gradually rose to the normal range with normalisation of PTH and ALP. She was discharged home on no treatment. The mother was found to have a large right inferior parathyroid adenoma, which was surgically removed. Post-operative hypocalcaemia was treated with oral calcium supplementation and calcitriol. When the serum calcium rose above 2.0mmol/L a stoss dose of 150,000units 25 OHvitamin D was administered, to correct the vitamin D deficient state. Vitamin D deficiency masked symptoms of long standing primary hyperparathyroidism in the mother by artificially reducing serum calcium. High levels of maternal PTH suppressed parathyroid function in twin 1, with severe clinical consequences and a very slow recovery requiring very large supplements. Twin 2 was entirely unaffected by the maternal vitamin D deficiency or hyperparathyroidism.

316

SOLID PHASE EXTRACTION OF PROSTAGLANDINS E_2, F_{2A} AND THEIR METABOLITES FROM BIOLOGICAL SAMPLES.

C. M. Mitchell [1], T. Richards [1], S. Hubbard [1], T. Welsh [1], S. Mesiano [1,2], P. K. Zarzycki [3], T. Zakar [1,2]

[1] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

[2] Obstetrics and Gynaecology, John Hunter Hospital, Newcastle, NSW, Australia

[3] Laboratory of Toxicology, Technical University of Koszalin, Poland

Prostaglandins (PGs) are present in many tissues of the body and function in a diverse range of biological processes. Before separation and quantification, PGs from biological samples must be extracted and purified [1]. We have undertaken systematic studies to establish a solid phase extraction (SPE) method for the purification of the biologically active prostaglandins PGE_2, $PGF_{2\alpha}$and the full range of their tissue metabolites. The SPE system was composed of an octadecylsilane stationary phase (C18, 40mg, 6ml Supelclean^{TM} cartridges, Supelco) and binary mobile phases of methanol and water 1%-100% (v/v). PGs were eluted in 100% methanol and dried, before TLC was performed and PG recovery determined by densitometry. Using the same optimised sequence of mobile phases, over 90% recoveries of PGE_2, 15-keto-PGE_2, PGAM, bicyclo-PGE_2, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, and PGFM standards were achieved. Recovery for PGEM, which is a chemically unstable prostaglandin, was 81%. This optimised procedure was then tested in biological samples. PG recovery was verified by the addition of $^3HPGE_2$ and $^3HPGF_{2\alpha}$to urine, plasma and placental tissue extract. Sample type determined recovery, rather than the individual PG, with urine giving the highest recovery (>78%) followed by placental tissue (>58%) and plasma (>34%). The addition of 1% formic acid to the biological sample dramatically increased the recovery of PGs to 88%-100% in all biological matrices. This suggests that formic acid diminishes interactions between biological matrix components and PGs in the samples. This methodology is capable of extracting and purifying a wide range of bioactive PGs and metabolites from complex biological materials. It may serve as a pre-purification step in a variety of applications that include downstream separation and quantification.

(1) Welsh, et al; J.Planar Chromatogr,16 (2003) 95-101.

317

FOLLISTATIN CHANGES SIGNIFICANTLY IN SPONTANEOUS LABOURING WOMEN.

K. M. Rae [1], K. G. Hollebone [2], M. S. Baylis [2], D. C. Clausen [3], V. Chetty [3], J. R. McFarlane [1]

[1] University of New England, Armidale, NSW, Australia

[2] Obstetrics Unit, Tamworth Base Hospital, Tamworth, NSW, Australia

[3] Pathology New England, Tamworth, NSW, Australia

Follistatin an activin binding protein has been isolated from human placenta, human foetal membranes and fluids and is increased in serum during late gestation. This study examines the effect of induction and stage of labour on maternal plasma concentrations of follistatin. Following normal pregnancy women were retrospectively assigned into 3 groups; spontaneously labouring patients (n=24), patients

induced by membrane rupture and IV syntocin (n=16), and caesarean section patients (n=9). Blood samples were taken at 38-40 weeks gestation and during a vaginal exam in labour. Follistatin was measured using rabbit antiserum (#204) raised against purified 35kDa bovine follistatin and human recombinant follistatin was used as the standard (1). Plasma concentrations of follistatin at 38-40 weeks of gestation were significantly different between groups with spontaneous labour patients higher than induced patients and the caesarean group. In the spontaneous group follistatin rose during labour peaking at 50 ± 10 ng/ml with >5cm of cervical dilation and declined to 20 ± 4 ng/ml at 24 hr post delivery. The induced patient group showed follistatin remaining relatively unchanged during labour and post delivery at 14 ± 3 ng/ml. In caesarean section patients follistatin concentrations at 24hr post surgery (5.3 ± 1.2 ng/ml) were no different from pre-surgery values and significantly lower than the other two groups. These results show that there is a rise in follistatin during labour only in those women undergoing spontaneous labour, suggesting the rise is triggered prior to the onset of labour and that follistatin may play an important role in parturition.

(1) O'Connor, AE, McFarlane, JR, Hayward, S, Yohkaichiya, T, Groome, NP and de Kretser, DM (1999). "Serum activin A and follistatin concentrations during human pregnancy: a cross-sectional and longitudinal study [In Process Citation]." Hum Reprod 14(3): 827-32.

318

REGULATION OF VOLTAGE-GATED K^+ CURRENTS OF RAT SOMATOTROPES BY SOMATOSTATIN RECEPTORS

S. Yang , C. Chen

Endocrine Cell Biology, Prince Henry's Institute, Clayton, VIC, Australia

The secretion of growth hormone (GH) is regulated by hypothalamic hormones GH-releasing hormone (GHRH) and somatotrophin releasing inhibitory factor or somatostatin (SRIF). SRIF inhibits GH secretion via specific somatostatin receptors (SSTRs). Five subtypes of SSTRs have been identified. SRIF increases voltage-gated K^+ currents, both transient outward (I_A) and delayed rectifying (I_K), through activation of Gi3 proteins in rat somatotropes (1) via unidentified SSTR subtype(s). In this study, we tested for a link between SSTRs and K^+ currents in the GH3 rat somatotrope cell line using specific SSTR agonists, L-797,591 ($SSTR_1$), L-797,976 ($SSTR_2$), L-797,778 ($SSTR_3$), L-803,087 ($SSTR_4$), and L-817,818 ($SSTR_5$>$SSTR_1$). All five SSTR mRNAs are detected in GH3 cells by RT-PCR, with dominant expression of the $SSTR_2$ gene. Nystatin-perforated whole-cell patch clamp configuration was employed to record voltage-gated K^+ currents with a holding potential of -70 mV in the presence of Ca^{2+} channel blocker, nifedipine, and Na^+ channel blocker, tetrodotoxin, in the bath solution. Activation of $SSTR_2$ and $SSTR_4$ with maximal dose of L-797,976 or L-803,087 increased voltage-gated K^+ currents without preference to I_A or I_K and abolished any further increase by SRIF. Activation of $SSTR_1$ and $SSTR_5$ with maximal dose of L-797,591 or L-817,818 increased the K^+ currents but SRIF evoked a further increase. Sub-maximal doses of $SSTR_1$ and $SSTR_5$ agonists did not increase K^+ currents and did not affect the response to subsequent SRIF administration. $SSTR_3$ analogue at maximal dose did not modify the K^+ currents and did not affect the K^+ current response to SRIF. These results indicate that $SSTR_2$ and $SSTR_4$ are involved in regulating voltage-gated K^+ currents by SRIF. Identifying which SSTR subtypes inhibit GH secretion is under investigation.

(1) Chen C. Gi3 protein mediates the increase in voltage-gated K+ currents by somatostatin on cultured ovine somatotrophs. Am J Physiol 275: E278-E284.

319

TIME-DEPENDENT EFFECTS OF GHRH AND GHRP-2 ON THE EXPRESSION RATES OF GH, GHRH-RECEPTOR, GHS-RECEPTOR, SOMATOSTATIN RECEPTORS (SST) AND PIT-1 AND THE SECRETION OF GH IN PRIMARY CULTURED OVINE PITUITARY CELLS

M. M. Hernandez , M. Yan , C. Chen

Endocrine Cell Biology, Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

GH releasing hormone (GHRH) and GH releasing peptide 2 (GHRP-2) stimulate GH secretion from somatotropes and elevate GH levels in vivo and in vitro. GHRH and GHRP treatment has been used for diagnosis and treatment of GH deficiency in adults and children. The mechanism of long-term GHRH and GHRP treatment is not clear. In particular, the molecular targets in somatotropes and the time-effect relationship are not known. Aiming to clarify this issue, we measured the mRNA levels of GH, Pit-1, GH secretagogues receptor (GHS-R, specific GHRP receptor), GHRH-R, sst-1 and sst-2 and accumulated GH secretion following 4, 10, 24 and 72 hours of GHRH (10^{-9} M)/GHRP-2 (10^{-8} M) treatment of ovine pituitary cells. Pituitary cells were obtained from a local abattoir, dissociated by enzymes and cultured in serum-containing medium for 2 days before treatment. GHRH/GHRP treatment for 10h or 24h increased the levels of GH mRNA expression and accumulated secretion in culture medium. The levels of Pit-1, GHS-R and GHRH-R mRNA were also increased by 10h or 24h treatment. The increase in levels of GH, GHS-R and GHRH-R mRNAs and GH concentration declined to control levels at 72h treatment. GHRH/GHRP-2 treatment for 10h, 24h and 72h increased the levels of sst-1 mRNA, whereas sst-2 mRNA was increased by 10h or 24h treatment, but levels had declined at 72h. These results indicate that GHRH and GHRP-2 are important regulators of pituitary GH expression and secretion. In addition to triggering immediate GH secretion, GHRH and GHRP-2 regulate the levels of GH, GHRH-R, GHS-R, Pit-1, sst-1 and sst-2 mRNAs in a time-dependent manner . The effects on somatotropes result in either a priming or a depriving modification of the cell function, leading to increased or decreased GH secretion. An extended time-effect relationship study and combined GHRH/GHRP and somatostatin treatment warrant further investigation.

320

THE ROLE OF THERMOGENESIS IN THE RESISTANCE TO DIET INDUCED OBESITY

B. J. Oldfield [1], M. J. Morris [2], C. Wuller [1]

[1] Howard Florey Institute, Melbourne, VIC, Australia

[2] Department of Pharmacology, University of Melbourne, Melbourne, VIC, Australia

It is well established that Sprague Dawley rats when exposed to a high fat diet exhibit a bimodal weight gain similar to that seen in human populations. There is a pervasive view that the different weight gain seen in obese prone (OP) and obese resistant (OR) rats reflects a genetically-determined difference in energy expenditure. In these experiments, rats were fed a high fat cafeteria diet and both thermogenic activity in BAT and physical activity were measured using telemetry. During the 15 weeks of the diet, OP rats consumed 23% more energy than OR rats but gained 93% more weight. Similarly, OR rats consumed 76% more energy than controls (fed chow) but gained 7% less weight. These discrepancies may be explained by the fact that the average BAT temperature was higher in the OR than the OP rats ($P<0.001$). Resting energy expenditure was greater in the OR group for most of the feeding regime but there was no difference in physical activity. Circulating levels of leptin were higher in the OP group which reflected levels of body fat. These data show that thermogenesis in BAT is elevated in OR rats and this may at least partly explain the ability of these animals to defend their body weight in the face of a high fat diet.

321

EXPRESSION OF MRNA FOR HORMONES REGULATING FEEDING RESPONSES IN THE NEONATAL PIG STOMACH

B. Zhu, M. J. Gresham, R. Newman, P. C. Wynn

Reprogen, Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia

The elucidation of mechanisms regulating suckling in neonatal pigs will lead to strategies to stimulate colostrum and milk intake in smaller pigs. Thus we have investigated the expression of key orexigenic (ghrelin) and anorexic (leptin) hormones and their receptors in the stomach of the piglet. The cDNA sequences for ghrelin and its receptor (GHS-R), and leptin and its receptor (Leptin-R) from the gene bank were used to design specific primers for gene amplification. Specific sequences (200 – 264 base pairs) were obtained using reverse-transcription-polymerase chain reaction (RT-PCR), as follows: cDNA synthesis at 50 ° C for 30min, 94 ° C for 2 min and 35 cycles of PCR amplication at 94 ° C for 1 min, 52 ° C for 1 min and 72 ° C for 1 min, then final extension for 10 min at 72 ° C. The sequences for ghrelin, leptin and leptin-R were obtained from the pyloric region of the stomach, while the GHS-R sequence was cloned from the hypothalamus. The products were cloned into the pCR Ò II-TOPO Ò vector (Invitrogen) and verified by dideoxy termination sequencing. Total RNA was extracted from the oesophageal, cardiac, fundic and pyloric glands of the stomach of 3-day old piglets using Tri-reagent (Sigma). Clone DNA (ghrelin, GHS-R, leptin, and leptin-R) was used as the control and the above RT-PCR method was employed to determine their expression. Ghrelin was identified in the cardiac and fundic glands while its receptor was apparent in all stomach regions. Similarly leptin receptor mRNA was expressed in all 4 glands, while leptin was expressed in the pyloric region. These results suggest that ghrelin and leptin of gastric origin are implicated in the regulation of hypothalamic satiety mechanisms in the 3-day-old piglet. The co-expression of ligand and homologous receptors in gastric tissue suggests that ghrelin and leptin act through a short-term negative feedback mechanism, the nature of which remains to be resolved.

GLUCOCORTICOID RECEPTOR EXPRESSION FROM THE 1A PROMOTER CORRELATES WITH T LYMPHOCYTE SENSITIVITY TO GLUCOCORTICOID-INDUCED CELL DEATH

T. J. Cole [1], J. F. Purton [3], J. A. Monk [1], D. R. Liddicoat [1,2], D. A. Bird [1], S. J. Richardson [1], D. I. Godfrey [2]

[1] Biochemistry & Molecular Biology, University of Melbourne, Parkville, VIC, Australia
[2] Immunology & Microbiology, University of Melbourne, Parkville, VIC, Australia
[3] Scripps Institute, San Diego, CA, United States

Synthetic glucocorticoid steroids, such as dexamethasone and prednisolone, are commonly used as potent anti-inflammatory agents during the clinical treatment of human trauma. A side-effect of these high doses is a major down-regulation of the immune system, particularly a massive death of T-cells, which can have a major impact on patient recovery and potential mortality. This pronounced T cell apoptosis, particularly in immature thymic T cells is potentially due to tissue-specific regulation of the glucocorticoid receptor (GR) gene. In mice, the GR gene is transcribed from five separate promoters designated: 1A, 1B, 1C, 1D, and 1E. Nearly all cells express GR from promoters 1B-1E, but activity of the 1A promoter has only been reported in the whole thymus or lymphocyte cell lines. To directly assess the role of GR promoter use in sensitivity to glucocorticoid-induced cell death (GICD) we have compared activity of the GR 1A promoter with glucocorticoid-sensitivity in different lymphocyte populations. We report that GR 1A promoter activity is restricted to thymocyte and peripheral lymphocyte populations. The relative level of expression of the 1A promoter to the 1B-1E promoters within a lymphocyte population was found to directly correlate with susceptibility to GC-induced cell death, with the extremely GC-sensitive $CD4^{+}CD8^{+}$ (DP) thymocytes having the highest levels of GR 1A promoter activity and the relatively GC-resistant $TCR^{+}CD24^{int/lo}$ thymocytes, and peripheral lymphocytes, having the lowest. DNA sequencing of the mouse GR 1A promoter revealed a putative glucocorticoid-response element (GRE). Glucocorticoid-sensitive lymphocytes were found to increase GR protein levels following treatment, while glucocorticoid-resistant lymphocytes did not. These data suggest that tissue-specific differences in GR promoter use are an origin of T cell sensitivity to GICD.

ACTIVIN A AND FOLLISTATIN RESPONSE IN ACUTE HEPATIC INFLAMMATION

S. Patella [1,2], K. L. Jones [1], K. Sebire [1], C. Stringer [2], W. Sievert [2], D. M. De Kretser [1], D. J. Phillips [1]

[1] MIRD, Monash University, Clayton, VIC, Australia
[2] Medicine, Monash University, Clayton, VIC, Australia

Activin A is known to respond to inflammatory mediators and form part of the inflammatory pathway. Additionally, activin A is known to profoundly influence liver biology, being an autocrine inhibitor of hepatocyte DNA synthesis and an inducer of hepatocyte apoptosis. Using a model of acute carbon tetrachloride induced liver inflammation, here we analyse the expression of activin A and follistatin in relation to other pro-inflammatory cytokines. Localised liver inflammation was induced in C57BL/6 mice by a single intraperitoneal injection of Carbon tetrachloride (CCl_4) mixed with olive oil. Animals were killed at various time points following CCl_4 administration up to 72 hours. Serum samples were taken for analysis of cytokine concentrations by ELISA. Expression of various gene products was determined by PCR analysis and histological analysis was performed on tissue samples. Histological analysis following CCl_4 administration revealed significant pathological changes 1 hour following intoxication. Peri-central and peri-portal hepatocytes were observed to undergo cell death, co-incident with lymphocytic infiltration, which continuing to 48 hours. By 72 hours, lymphocytic infiltration had persisted, but damaged hepatocytes had been replaced. Activin A immunohistochemistry revealed localisation to necrotic areas at early time points, but at later time points to lobular hepatocytes. Serum and tissue activin A and follistatin levels increased significantly 36 hours after CCl_4 administration. These elevations coincided with elevations in other pro-inflammatory cytokines such as TNF alpha, IL1 and IL6. PCR analysis revealed decreases in activin A expression 24 hours following CCl_4 administration, consistent with hepatocyte regenerative responses. Previous analysis of activin A and follistatin release using models of systemic inflammation have demonstrated elevations in serum activin A extremely early following exposure to inflammatory stimului. Here we demonstrate in a model of organ specific inflammation, serum and tissue elevations in activin A occur some time following exposure to the inflammatory stimulus but still coincident with other pro-inflammatory mediators. The results presented here demonstrate that while the kinetics of activin A and follistatin release may differ dependant on the nature of the inflammatory stimulus, activin A release appears coincident with other pro-inflammatory cytokines.

324

EXPRESSION OF TYPE I & TYPE II ALVEOLAR EPITHELIAL CELL MARKER GENES IN THE DEVELOPING LUNG OF GR-/- MICE

D. A. Bird [1], S. B. Hooper [2], R. Mollard [3], T. J. Cole [1]

[1] Biochemistry & Molecular Biology, Melbourne University, Parkville, VIC, Australia
[2] Department of Physiology, Monash University, Clayton, VIC, Australia
[3] Institute of Reproduction and Development, Monash University, Clayton, VIC, Australia

Glucocorticoids (GCs) regulate a range of important physiological processes such as stimulating gluconeogenesis, suppressing inflammatory responses, and in the developing mammalian embryo promote the maturation of the lung. Synthetic GCs such as betamethasone have been widely used clinically with preterm infants to counter the effects of respiratory distress syndrome. Recently, our laboratory has studied foetal lung tissue from mice homozygous for a gene-targeted null mutation of the Glucocorticoid Receptor (GR). GR null foetal mice prior to birth have increased lung weight and DNA content, are condensed and hypercellular, with reduced septal thinning leading to a 6-fold increase in the airway-capillary diffusion distance. Expression of mRNA for the type II alveolar epithelial cell (AEC) surfactant proteins A & C was reduced by 50%. Analysis by electron microscopy revealed that the proportion of type II cells was increased by ~30%, while the proportion of type I cells was significantly reduced by 50%. Expression of type I and type II AEC marker genes have been quantified in foetal lung tissue at Day 16.5, 17.5, and 18.5 post coitum. These results demonstrate that complete differentiation of type I AECs is GR-dependent, but GR is not obligatory for differentiation of type II AECs in the developing mouse lung just prior to birth.

325

CHARACTERISATION OF THE ANDROGEN RECEPTOR PATHWAY IN EARLY EMBRYONIC DEVELOPMENT

D. R. Goldman [1], J. R. Morrison [2]

[1] MIRD, Monash University, Clayton, VIC, Australia
[2] CopyRat, Pty. Ltd., Clayton, VIC, Australia

The balance of androgens in the foetus is known to be essential for normal embryonic and adult development and is also thought to establish an individual's susceptibility to carcinogenesis. Little is known about androgen receptor (AR) pathway signalling in early embryonic formation or the switches that occur to promote expression of the pathway at important developmental phases. My current research aims to characterise the AR pathway in mouse ES cells and early embryonic stages. Initially, I confirmed the expression of AR message in mouse ES cells using RT-PCR, however this does not equivocally demonstrate the functionality of the AR pathway. In order to do so, the following techniques are being applied: (1) Radioactive microarray systems that demonstrate the relative expression of AR pathway-related and prostate cancer genes in untreated ES cells compared with those treated for 24 hours with either 10^{-10}M testosterone (T) or 10^{-10}M dihydrotestosterone (DHT). This will help to establish the functionality of the AR pathway and determine if, at this early embryonic stage, it is capable of ligand processing. (2) Co-immunoprecipitation/western assays will be used to detect AR protein in treated and untreated ES cells, and its localisation will be detected by immunohistochemistry. (3) Fluorescent reporter assays have been established where expression is under the control of a mouse mammary tumour long terminal repeat (MMTV-LTR) promoter, which

acts as an androgen response element (ARE). Detection of ARE-GFP vector expression will be compared between T or DHT treated and untreated ES cells. (4) Differentiation studies will be used to examine the response of ES cells to androgen stimulation and to characterise the AR pathway in early embryonic development via quantitative PCR or radioactive microarray systems in order to observe variation in the expression of androgen responsive genes.

326

WESTERN BLOT CHARACTERISATION OF THE HUMAN MYOMETRIUM IN LABOUR

D. A. MacIntyre , R. Smith , E. C. Chan

Mothers and Babies Research Centre, Hunter Medical Research Institute, Callaghan, NSW, Australia

Parturition is the process by which the human uterus transforms from a quiescent state into an active state to expel the fetus through a softened cervix. The mechanisms that regulate this process remain largely unknown. RNA subtraction studies using suppression subtractive hybridization published by our laboratory (1) have identified a number of genes that are upregulated with the onset of labour. To extend these studies into the myometrial proteome, western blot analysis was used to investigate the expression of actin (α, β), nucleophosmin (B23), elongation factor Tu (EF-Tu), fibronectin, interleukin-8 (IL-8) and manganese superoxide dismutase (MnSOD) in the human myometrium at term. GAPDH was also included as a constitutively expressed protein. All antibodies were purchased from Santa Cruz Biotechnology, Inc. Biopsies of the lower uterine segment of women in labour (n=3) and not in labour (n=5) were extracted in 2D electrophoresis lysis buffer (7M Urea, 2M Thiourea, 30mM Tris, 4% CHAPS, pH 8.5). A total of 10μg of protein was resolved on NuPAGE® pre-cast BIS/Tris or Tris/Acetate polyacrylamide gels (Invitrogen), under reducing conditions. Following this, proteins were transferred to nitrocellulose membrane and probed with primary and secondary (HRP-conjugated) antibodies. Detection was performed using ECL™ Western blotting Detection Reagents (Amersham Biosciences) and Supersignal® West Dura Extended Duration Substrate (Pierce). The results showed that all of the proteins of interest were expressed in the myometrium. A full understanding of myometrial function during labour will require data on both mRNA and protein change in the tissue. This work describes initial efforts to characterise the myometrial proteome in the crucial transition from non-labouring to labouring.

(1) Chan EC, et al. 2002 J Clin Endocrinol Metab 87 (6) 2435-2441

327

PROSTAGLANDIN F AND E SYNTHASE AND RECEPTOR EXPRESSION IN THE AMNION AND CERVIX OF THE PARTURIENT EWE

H. K. Palliser [1], J. J. Hirst [1], G. E. Rice [2], G. T. Ooi [3], N. L. Dellios [1], R. M. Escalona [3], I. R. Young [1]

[1] Physiology, Monash University, Clayton, VIC, Australia
[2] Obstetrics & Gynecology, Mercy Hospital for Women, East Melbourne, VIC, Australia
[3] Reproductive Endocrinology, PHIMR, Clayton, VIC, Australia

Background: Prostaglandins (PG) E_2 and $F_{2\alpha}$ play a pivotal role in cervical dilation, ripening and membrane rupture during labour. The mechanisms regulating the production of specific PGs and their actions are largely unknown, however these pathways are likely targets of interventions that block preterm delivery. The aims of this study were to determine PGH synthase-2, PGF synthase, PGE synthase and PG receptor (FP and EP2-4) mRNA expression in fetal membranes and cervical tissues following labour onset. Methods: Two groups of 4 pregnant ewes were killed during spontaneous or dexamethasone-induced labour. A third group of saline-treated, non-labouring ewes served as controls. mRNA encoding PG synthases and receptors were measured by real time PCR in mid-section cervix and amnion. The veno-arterial difference of plasma PGs was assayed to determine net PG synthetic activity in gestational tissues. Results: PGHS-2 mRNA expression decreased in the amnion of spontaneous and induced labour ewes ($P<0.05$) and increased in the cervix of spontaneous labour ewes ($P<0.05$). PGFS mRNA expression decreased in the amnion of the induced labour ewes ($P<0.05$). FP mRNA expression decreased in the amnion of induced labour and the cervix of the spontaneous labour ewes ($P<0.05$). There was a significant increase in EP3 mRNA expression in the cervix of induced labour ewes. No labour-associated change was found in PGES or EP2 mRNA expression in either tissue. Conclusion: The decrease found in PGHS-2 expression in the amnion of labouring ewes is a novel finding. PGs acting on the amnion during labour may be derived from other tissues such as the placenta. The decrease in PGFS and FP expression in the amnion of induced-labour ewes correlates with decreased $PGF_{2\alpha}$ production and action in this tissue. The increase in PGHS-2 and EP3 expression in the cervix is consistent with the documented action of PGE_2 in this tissue.

328

HUR INTERACTIONS WITH THE 3'-UTR OF EGF RECEPTOR (EGF-R) AND C-*ERB*-B2 (HER-2) IN HUMAN BREAST CANCER.

M. R. Epis [1,2], E. Hollams [1,2], A. M. Thomson [1,2], D. J. Beveridge [1,2], K. M. Giles [1,2], P. J. Leedman [1,2]

[1] Centre for Medical Research, WAIMR, University of WA, Perth, WA, Australia
[2] School of Medicine and Pharmacology, University of WA, Perth, WA, Australia

The EGF-R and HER-2 oncogenes are significantly over-expressed in human breast cancers (BCa) and are major therapeutic targets. While rates of mRNA transcription are important in many cell systems, there are numerous examples of genes that are regulated at the post transcriptional (mRNA decay) level. Our studies have identified novel U-rich *cis*-acting elements in the 3'-untranslated region (3'-UTR) of EGF-R and HER-2 mRNAs. Here, we aimed to characterise some of the key RNA/protein interactions targeting these *cis*-elements. Interestingly, there is mounting evidence for a critical role for HuR as a modulator of cell growth in colon and brain cancer. In particular, we wished

to investigate if HuR, a member of the Hu family of RNA binding proteins, targeted either of these regions, and if so, the functional effects. We stably under and over-expressed HuR in MDA468 and SkBr3 BCa cell lines, and found that alteration of the HuR levels lead to a profound change in EGF-R protein and mRNA levels. Specifically, reduction in HuR levels dramatically decreased EGF-R protein levels in MDA468 cells. Similarly, HER-2 was also significantly reduced in HuR depleted SkBr3 cells. Using an IP-RT-PCR assay in MDA468 and SkBr3 cells, we found that HuR was closely associated with EGF-R and HER-2 mRNAs. Gel shift assays confirmed binding of HuR to both the EGF-R and HER-2 *cis*-elements. In summary, these studies indicate a novel role for HuR in the regulation of EGF-R and HER-2 gene expression in human BCa cells. These data have important implications for the functional role of this protein and suggest it could be a potential therapeutic target.

329

A FOUR YEAR AUDIT OF SEVERE HYPOTONIC HYPONATRAEMIA AT A GENERAL HOSPITAL

M. Grossmann [1,2], **P. S. Hamblin** [1,2], **R. Wong** [2]

[1] ***EDMU, Alfred Hospital, Melbourne, VIC, Australia***

[2] ***Endocrinology, Western Hospital, Footscray, VIC, Australia***

Aims: (1) to define the major causes of severe hypotonic hyponatraemia (SHH, defined as serum sodium < 120 mmol/L) presenting to a general hospital; (2) to assess the adequacy of the diagnostic workup of these patients; (3) to determine treatment outcomes.

Methods: Patients of Western Hospital were identified by biochemistry search from January 2000 to December 2003 and data obtained from patient medical records.

Results: Of 159 admissions with SHH, 60 were male and 99 female. Mean age was 70.3 years (range 32-98). Main presenting complaints were lethargy/weakness/falls (38%), acute brain syndrome (35%), and gastrointestinal symptoms (27%). Urinary osmolality and sodium was performed in 106 patients, but only 10 were neither dehydrated, nor on diuretics nor receiving saline hydration. TSH measurement was diagnostic in one of 91 patients and cortisol in one of 52 patients. CT brain was performed in 63 patients and normal in all but 3 patients with clinical stroke. SHH was considered to be due to thiazide diuretics in 47 patients (30%), dehydration in 29 (18%), SIADH in 28 (18%, although strict criteria[1] were met in only 3), ethanol binge in 24 (15%), multifactorial in 24 (14%), CCF in 4, and endocrine causes in 3. Patients with thiazide-induced hyponatraemia were predominantly female (85%) and had lower serum potassium (3.4+0.6 vs. 3.9+0.9). SIADH was due to medications in 72%, with SSRIs and ACE inhibitors most commonly implicated. Patients with SIADH had higher urine osmolality (447+155 vs. 347+161) and urine sodium concentrations (75+33 vs. 48+38). Treatment-induced osmotic demyelination occurred in 2 of 5 patients with a rapid (>20 mmol/1st 24 hours) sodium rise. Overall mortality was 14% and not related to sodium level at presentation, but to comorbidities. Median length of hospital stay was 7 days (range 1-100).

Conclusions: Hyponatraemia is a common clinical problem that is often investigated suboptimally. Even in fluid-resuscitated patients, high urinary osmolality/sodium excretion may identify SIADH. The high proportion of medication-induced SHH emphazises the importance of sodium measurements especially after initiation of thiazide diuretics. Avoidance of overly rapid sodium correction is critical to avoid osmotic demyelination.

(1) S.Kumar et al., Lancet 352:220, 1998

MATERNAL EXPOSURE TO DEXAMETHASONE OR CORTISOL IN EARLY PREGNANCY CAUSES FASTING HYPERGLYCAEMIA AND HYPERINSULINAEMIA IN ADULT MALE OFFSPRING IN SHEEP.

M. J. De Blasio [1], A. Jefferies [2], K. Moritz [2], M. Dodic [2], M. Wintour-Coghlan [2], J. S. Robinson [1], J. A. Owens [1]

[1] Department Of Obstetrics And Gynaecology, University Of Adelaide, Adelaide, SA, Australia
[2] Department of Physiology, Monash University, Clayton, VIC, Australia

Epidemiological studies have demonstrated that fetal exposure to an adverse intra-uterine environment at critical periods during development can increase the risk of developing adult-onset cardiovascular and metabolic disease. Pregnant sheep were exposed to saline, dexamethasone (0.48 mg/hour) or cortisol (5 mg/hour), for 48 hours from 26 to 28 days gestation, then glucose tolerance and whole body insulin sensitivity assessed by hyperinsulinaemic euglycaemic clamp in their adult male offspring at ~ 4.5 years of age. Glucose tolerance was calculated as the area under the curve, after intravenous administration of a bolus of glucose (0.25g/kg body weight), and insulin secretion was measured as the insulin area under the curve of the response to the glucose challenge. Birth weight was reduced in the dexamethasone (4.1 + 0.2 kg) compared with the cortisol (4.8 + 0.2 kg) group ($p<0.05$), but these were not different to the saline (4.6 + 0.3 kg) group ($p=0.12$). Fasting plasma glucose was increased in the dexamethasone (3.7 + 0.2 mmol/l, $p=0.06$) and cortisol (3.8 + 0.2 mmol/l, $p<0.05$) groups compared to the saline (3.2 + 0.1 mmol/l) group. Fasting plasma insulin was increased in the dexamethasone (7.3 + 0.9 m U/ml, $p=0.026$) and cortisol (10.9 + 1.0 m U/ml, $p=0.002$) groups compared to the saline (4.2 + 0.7 m U/ml) group, but the fasting insulin/glucose ratio was not altered. Similarly, the basal and stimulated insulin disposition indices, the product of fasting plasma insulin or insulin secretion and insulin sensitivity, were not altered by treatment. The area under the glucose curve (AUC glucose) was reduced in the dexamethasone (343 + 32 mmol.min/l, $p<0.05$) group compared with the saline (484 + 60 mmol.min/l) and cortisol (480 + 39 mmol.min/l) groups. In conclusion, dexamethasone or cortisol exposure during early gestation causes fasting hyperglycaemia and hyperinsulinaemia in adult male offspring, but does not impair glucose tolerance, insulin secretion or sensitivity. Because of the major role of the liver in determining fasting glycaemia, this suggests early gestational steroid exposure may adversely program hepatic glucose production and insulin sensitivity in postnatal life, predisposing to development of metabolic disease with ageing.