Introduction

2001

I am delighted to present for the first time in some years a specific Research Report section of our Annual Report. This is a more comprehensive account of research from the Baker laboratories than it has been possible to put in our smaller and admittedly more readable report in recent years. We hope it will be of value particularly to scientists and others interested in learning about Baker research in greater depth.

The report shows an increasing number of high impact publications from the Institute and lists many achievements of our staff. As always at the Baker there is strong basis in experimental research but also extensive applications in the clinic and beyond to the community.

We are most grateful to our collaborators and supporters, particularly Alfred Hospital and Monash University colleagues, the donors, patients and volunteers who all contributed to make this work possible.

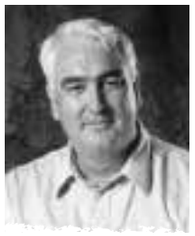
Professor Gary Jennings
DIRECTOR

Coronary Disease & Vascular Division

Coronary Disease and Vascular Division

Thrombosis Group

Hazel & Pip Appel Vascular Biology



HEAD
Michael C Berndt
BScHons, PhD (Qld)

SENIOR SCIENTIFIC
Robert K Andrews
BScHons, PhD (Qld)
Yang Shen MMedScHons (China), PhD (Adelaide)
Elizabeth E Gardiner BScHons, PhD (Monash)
PROFESSIONAL & TECHNICAL
Cheryl Berndt CertLabTech (Sydney)
Carmen Llerena AssocDipLabTech (Peninsular TAFE)
Andrea Aprico BScHons (Monash)
Patrizia Novello BScHons (LaTrobe)
Catherine Upton BScHons (Monash)

Research projects

Our research looks at the role of platelets in arterial thrombosis. Adhesion of platelets after atherosclerotic plaque rupture or activation by high shear stresses at sites of arterial stenosis may result in occlusive thrombus. In either scenario, the events are mediated by the platelet adhesion receptor, the GP Ib-IX-V complex, which binds von Willebrand factor (vWF).

Functional analysis of the C-terminal flanking sequence of platelet glycoprotein Ib using canine-human chimeras.
Yang Shen, Andrea Aprico, Elizabeth E. Gardiner, Michael C. Berndt, & Robert K. Andrews.

Platelet glycoproteinIb-IX-V (GPIb-IX-V) mediates adhesion to von Willebrand factor (vWF) in (patho)physiological thrombus formation. vWF binds the N-terminal 282 residues of GPIb, consisting of an N-terminal flank (His1-Ile35), 7 leucine-rich repeats (Leu36-Ala200), a C-terminal flank (Phe201-Gly268), and a sulfated tyrosine sequence (Asp269-Glu282). Previously, binding sites for functional anti-GPIb antibodies were mapped to individual domains of canine-human chimaeras of GPIb expressed on Chinese hamster ovary cells. Leucine-rich repeats 2 to 4 were required for optimal vWF recognition under static or flow conditions.

Using novel canine-human chimaeras dissecting the C-terminal flank, it is now demonstrated that:

- Phe201-Glu225 contains the epitope for AP1, an anti-GPIb monoclonal antibody that inhibits both ristocetin- and botrocetin-dependent vWF binding

- VM16d, an antibody that preferentially inhibits botrocetin-dependent vWF binding, recognizes the sequence Val226-Gly268, surrounding Cys248, which forms a disulfide-bond with Cys209

- vWF binding to chimaeric GPIb is comparable to wild-type in 2 chimaeras in which the sixth leucine-rich repeat was of the same species as the first disulfide loop (Phe201-Cys248) of the C-terminal flank, suggesting an interaction between these domains may be important for optimal vWF binding

- replacing the C-terminal flank second disulfide loop (Asp249-Gly268) in human GPIb with the corresponding canine sequence enhanced vWF binding under static and flow conditions. This finding provided the first evidence for a gain-of-function phenotype associated with the second loop of the C-terminal flank.

Regulation of P-selectin binding to the neutrophil P-selectin counter-receptor, P-selectin glycoprotein ligand-1, by neutrophil elastase and cathepsin G.
Elizabeth E. Gardiner, Mariagrazia De Luca, Tracy McNally, Robert K. Andrews & Michael C. Berndt.

In the inflammatory response, leukocyte rolling before adhesion and transmigration through the blood vessel wall is mediated by specific cell surface adhesion receptors. Neutrophil rolling involves the interaction of P-selectin expressed on activated endothelium and its counter-receptor on neutrophils, P-selectin glycoprotein ligand-1 (PSGL-1).

We found that binding of P-selectin to neutrophils is lost under conditions that cause the release of proteinases from neutrophil primary granules. Treatment of neutrophils with the granule

proteinases cathepsin G and elastase rapidly abolished their capacity to bind P-selectin. This inactivation corresponded to loss of the N-terminal domain of PSGL-1, as assessed by Western blot analysis. A loss of intact PSGL-1 protein from the surfaces of neutrophils after the induction of degranulation was also detected by Western blot analysis.

Cathepsin G initially cleaved near the PSGL-1 N-terminus, whereas neutrophil elastase predominantly cleaved at a more C-terminal site within the protein mucin core. Consistent with this, cathepsin G cleaved a synthetic peptide based on the PSGL-1 N-terminus between Tyr-7/Leu-8. Under conditions producing neutrophil degranulation in incubations containing mixtures of platelets and neutrophils PSGL-1, but not P-selectin, was lost from platelet-neutrophil lysates. Cathepsin G- or neutrophil elastase-mediated PSGL-1 proteolysis may constitute an autocrine mechanism for down-regulation of neutrophil adhesion to P-selectin.

Interaction of calmodulin with the cytoplasmic domain of the platelet membrane glycoprotein Ib-IX-V complex.
Robert K. Andrews & Michael C. Berndt.

Engagement of platelet membrane glycoprotein (GP) Ib-IX-V by von Willebrand factor triggers Ca++-dependent activation of GPIIb-IIIa, resulting in (patho)physiological thrombus formation. Based on bioinformatic searches, we found that the cytoplasmic domain of GPIb-IX-V associates with cytosolic calmodulin. First, an anti-GPIb antibody co-immunoprecipitated GPIb-IX and calmodulin from platelet lysates. Following platelet stimulation, calmodulin dissociated from GPIb-IX and, like the GPIb-IX-associated proteins 14-3-3 and p85, redistributed to the activated cytoskeleton. Second, a synthetic peptide based on the cytoplasmic sequence of GPIb, Arg149-Leu167 affinity-isolated calmodulin from platelet cytosol in the presence of Ca++ as confirmed by comigration with bovine calmodulin on sodium dodecyl sulfate-polyacrylamide gels, by sequence analysis, and by immunoreactivity with the use of an anti-calmodulin antibody. The membrane-proximal GPIb sequence was analogous to a previously reported calmodulin-binding sequence in the leukocyte adhesion receptor, L-selectin. In addition, the cytoplasmic sequence of GPV, Asp529-Gly544, was analogous to a calmodulin-binding IQ motif within the 1c subunit of L-type Ca++ channels. Calmodulin co-immunoprecipitated with GPV from resting platelet lysates, but was dissociated in stimulated platelets. A GPV-related synthetic peptide also bound calmodulin and induced a Ca++-dependent shift on nondenaturing gels. Together, these results suggest separate regions of GPIb-IX-V can directly bind calmodulin, and this novel interaction potentially regulates aspects of GPIb-IX-V-dependent platelet activation.

A novel viper venom metalloproteinase, alborhagin, is an agonist at the platelet collagen receptor GPVI.
Robert K. Andrews, Elizabeth E. Gardiner, A. Ian Smith & Michael C. Berndt.

The interaction of platelet membrane glycoprotein VI (GPVI) with collagen can initiate (patho)physiological thrombus formation. The viper venom C-type lectin family proteins convulxin and alboaggregin-A activate platelets by interacting with GPVI. In this study, we isolated alborhagin from white-lipped tree viper (Trimeresurus albolabris) venom, because it is functionally related to convulxin in that it activates platelets, but is structurally different, being related to venom metalloproteinases.

Alborhagin-induced platelet aggregation (EC50, <7.5 µg/ml) was inhibited by an anti-IIb3 antibody, CRC64, and the Src family kinase inhibitor PP1, suggesting that alborhagin activates platelets, leading to IIb3-dependent aggregation. Additional evidence suggested that, like convulxin, alborhagin activated platelets by a mechanism involving GPVI. First, alborhagin- and convulxin-treated platelets showed a similar tyrosine phosphorylation pattern, including a similar level of phospholipase C2 phosphorylation. Second, alborhagin induced GPVI-dependent responses in GPVI-transfected K562 and Jurkat cells. Third, alborhagin-dependent aggregation of mouse platelets was inhibited by the anti-GPVI monoclonal antibody, JAQ1. Alborhagin had minimal effect on convulxin binding to GPVI-expressing cells, indicating that these venom proteins may recognize distinct binding sites. Characterization of alborhagin as a GPVI agonist that is structurally distinct from convulxin demonstrates the versatility of snake venom toxins and provides a novel probe for GPVI-dependent platelet activation.

Grants and other funding

Ramaciotti special initiative grant to establish a dedicated proteomics and genomics facility

2001, $1,000,000

Welcome Equipment Grant
J Gorman, P Colman, N Hoogenraad, AI Smith and MC Berndt, 2001, $119,800

Molecular dissection of von Willebrand factor function
MC Berndt, National Heart Foundation of Australia, 2001-2002, $72,600

Identification of specific residues within adhesive domains on the platelet membrane glycoprotein Ib-IX-V complex that regulate thrombosis at high shear
RK Andrews, Y Shen and MC Berndt, National Heart Foundation of Australia, $90,000

Visiting scientists

Prof Jose A Lopez, Baylor College of Medicine, USA
Dr Chris Brown, University of Calgary, Canada

Presentations

11th International Conference on Second Messengers and Phosphoproteins, Melbourne

4th Australian Peptide Conference, Lindeman Island, Australia (Invited speaker)

3rd Annual Scientific Meeting of the Haematology Society of Australia and New Zealand, Brisbane, Australia (Invited speaker)

American Society of Hematology Meeting, Orlando, USA

XVIIIth Congress of the International Society on Thrombosis and Haemostasis, Paris, France (Invited speaker)

Australian Vascular Biology Society Annual Meeting, Noosa, Australia (Invited speaker)

Students

Honours
Lynley Moore

Vascular, Lipoprotein & Metabolism Group

Cell Biology

HEAD
Alex Bobik
BPharm (VIC),
MSc, PhD (Sydney)

SENIOR SCIENTIFIC
Alex Agrotis BScHons, PhD (Monash)
PROFESSIONAL & TECHNICAL
Peter Kanellakis BSc (Monash)
Giovanna Di Vitto BScHons (Melbourne)
Gina Kostolias BScHons (LaTrobe)

Research projects

gp91phox-Dependent NAD(P)H oxidase is highly expressed in human atherosclerotic lesions and regulated by transforming growth factor-beta and Interferon-gamma.
N Kalinina, A Agrotis, E Tararak, Y Antropova, P Kanellakis, O Ilyinskaya & A Bobik.

The superoxide anion-producing NAD(P)H oxidase(s) have been implicated in the pathogenesis of atherosclerosis. However, in severe human lesions, only p22phox has been identified and shown to be highly upregulated.

We examined the expression of cytochrome b558-dependent NAD(P)H oxidase-phox peptides in intimal smooth muscle cells (iSMCs) and macrophages of human aortic fibrofatty lesions and their regulation by cytokines. In normal intima and fatty streaks, about 25% of the iSMC expressed p22phox and gp91phox. Macrophages also expressed low levels of p47phox and p67phox. In fibrofatty

lesions, most of the iSMCs expressed p22phox and gp91phox, and p67phox was also detected.

Macrophages and macrophage-derived foam cells expressed all four phox subunits which were highly up-regulated by TGF-beta1 and IFN-gamma in cultured THP-1 monocytes. Primary cultures of aortic iSMCs also expressed the four phox mRNAs, with gp91phox and p67phox being up-regulated by IFN-gamma and TGF-beta1.Thus, in aortic fibrofatty lesions, cytokine regulated cytochrome b558-dependent NAD(P)H oxidase expressed by iSMCs and macrophages can contribute to superoxide production.

Transforming growth factor-beta-dependent Smad signaling is impaired in smooth muscle within macrophage-rich regions of human atherosclerotic fibrofatty lesions: A contributing factor to lesion instability?
N Kalinina, A Agrotis, E Tararak, O Ilyinskaya & A Bobik.

Transforming growth factor-beta (TGF-beta) isoforms and their signaling receptors are strongly expressed by smooth muscle cells (SMCs) and macrophages (Mphs) within the fibrofatty lesions (FFL) in atherosclerotic vessels. They can alter lesion characteristics via specific Smad transcription factors, activating a large variety of genes including those encoding collagens, plasminogen activator inhibitor-1 and the cyclin-dependent kinase (CDK) inhibitor p21(WAF), which protects against apoptosis.
We examined whether SMCs and Mphs in FFLs responded to TGF-beta.

The expression of Smads-2, -3 and -4 and CDK inhibitors in normal aortic intima and FFLs of human aortas were determined by immunohistochemistry. Their splice variants were examined by analysis of cDNA fragments generated by RT-PCR.

Expression of all three Smad transcription factors was high in SMCs from fibrous regions of FFLs, but absent from SMCs co-localising with dense populations of Mphs and Mph-derived foam cells. However, Mphs and Mph-derived foam cells in such regions expressed high levels of the three Smads and also the p21(WAF) peptide.

The major Smad-4 mRNA splice variant expressed by the SMCs and Mphs was its most active peptide, encoded by 11 exons. The major splice variants of Smads -2 and -3 (relative abundances >90%) encoded peptides capable of initiating TGF-beta mediated gene transcription. The lack of expression of Smads by SMCs within Mph-rich regions of FFLs would impair stabilisation of these regions by the production of a collagen-rich extracellular matrix.

Development of gene therapy approaches to reverse pathological left ventricular hypertrophy in experimental using adenoviruses.
A Bobik, A Agrotis, P Kanellakis & R Hannan.

Left ventricular hypertrophy (LVH) is a major risk factor for the development of congestive heart failure (HF) in people with high blood pressure. Despite antihypertensive treatments for LVH, progression to HF continutes to increase. Our studies were designed to increase understanding of the pathological mechanisms involved in the progression of LVH to HF and to devise targeted, novel, gene therapy approaches to reverse this progression.

We developed a rat model of hypertension by producing an aortic coarctation between the two renal arteries which caused systolic blood pressure rise from 115 to 175 mmHg after two days, by which time, LVH was apparent – myocyte size had increased by 15% and left ventricular interstitial collagen had increased nearly 4-fold.

Our gene therapy studies used adenoviruses expressing a truncated transforming growth factor-beta (TGF-beta) type II receptor which acted as a dominant negative mutant antagonist. We developed a keyhole surgical technique to deliver the viruses via intra-cardiac injection during ligation of the aorta at its trunk for 10 seconds. We are now assessing the effectiveness of inhibiting TGF-beta signaling on the fibrosis that develops in the hypertensive animals.

Experimental rupture of atherosclerotic lesions initially increases distal vascular resistance via constrictor mechanisms.
AJ Taylor, A Bobik, MC Berndt, P Kanellakis, D Ramsey and G Jennings.

Rupture of atherosclerotic lesions, resulting in localised thrombi and marked falls in distal blood flow, is a pivotal event in unstable coronary syndromes. We tested the hypothesis that following lesion rupture, vasoconstrictor mechanisms are major

contributors to the increased distal microvascular resistance that is responsible for much of the interruption in blood flow.

The endothelium was removed from the left iliac artery of cholesterol-fed rabbits to induce angiographically severe atherosclerotic lesions. After disrupting the lesions with a stiff wire, we measured distal blood flow and pressure, capillary patency in the distal vascular bed and the response to the vasodilators adenosine, nitroprusside and glyceryl trinitrate (GTN). Mean flow decreased from 5.04 ± 1.21 to 1.23 ± 0.37 ml/min (P<0.005) and calculated distal vascular resistance rose from 17.5± 2.9 to 37.9 ± 6.4 mmHg.min/ml (P<0.005). Lesion rupture did not significantly affect capillary patency in the distal muscular bed, and embolised thrombi were rare in capillaries (<1%).

The early rise in distal microvascular resistance was normalised by adenosine or the NO-donor nitroprusside, but not GTN. A component of the elevated vascular resistance appeared to be mediated by serotonin, released from platelets. Therapeutic targeting of the microvasculature should improve reperfusion in acute coronary syndromes.

Smooth muscle cell phenotype-specific repression of gene transcription is associated with promoter CpG methylation.
Alex Agrotis, Giovanna Di Vitto, Gina Kostolias & Alex Bobik.

Heterogeneity of arterial smooth muscle cells (SMCs) is thought to contribute to the formation of intimal lesions in human vascular diseases and pathologies such as atherosclerosis, vein-graft failure after bypass surgery, and restenosis after angioplasty. Our aim was to examine the basis for this heterogeneity, and the factors that contribute to intimal SMC expansion by examining the expression of fibroblast growth factor (FGF)-7, the fibroblast growth factor receptor (FGFR)-3, and the p22phox subunit of NADH/NAD(P)H oxidase genes in epithelioid and spindle rat SMC clones.

FGFR-3 and p22phox mRNA were strongly expressed only by epithelioid SMCs, while FGF-7 mRNA expression was abundantly expressed only by the spindle SMC clones. FGF-7 expression in the epithelioid clones increased in the presence of the DNA methylation inhibitor, 5-aza-2'-deoxycytidine (AZA), and the histone deacetylase inhibitor, Trichostatin A. Exposure of spindle SMCs to AZA led to the expression of FGFR-3 and p22phox.

Based on these results, we assessed the frequency of methylated cytosines near the transcriptional start sites of the genes. The spindle FGFR-3 and p22phox promoters and the epithelioid FGF-7 promoter showed extensive cytosine methylation. Our findings suggest that promoter CpG methylation is involved in both SMC heterogeneity and the regulation of SMC gene transcription contributing to the formation of intimal lesions.

Novel mitogenic fibroblast growth factor receptor/ligand expression in smooth muscle cells: Implications for neointima development.
Alex Agrotis, Gina Kostolias, Giovanna Di Vitto & Alex Bobik.

Fibroblast growth factor-2 (FGF-2) and fibroblast growth factor receptor-1 (FGFR-1) have been implicated in smooth muscle cell (SMC) proliferation within the media of the balloon catheter injured rat carotid artery. Other members of these protein families may also contribute to the SMC proliferation that occurs within the neointima. The aim of this study was to identity of these novel FGFs/FGFRs.

Using RT-PCR and nucleotide sequencing, we determined the expression profile of high affinity FGFRs and their FGF ligands, in the phenotypically distinct epithelioid and spindle SMC clones present in the neointimas of rat carotid arteries and aortas. Both cell types expressed spliced isoforms of FGFR-1 and FGFR-2, whilst FGFR-3 isoforms were expressed only by epithelioid clones. Significantly, we demonstrated for the first time that two novel, closely-related members of the FGF superfamily, FGF-9 and FGF-16, were expressed by SMCs. FGF-9 was expressed by most clones of either phenotype, whereas FGF-16 was expressed only by epithelioid SMCs. Both FGF-9 and FGF-16 were mitogenic for both phenotypes, most likely via activation of the MAP kinase pathway.

Functional significance of neointimal expression of the novel secreted mitogenic fibroblast growth factor, FGF-9, and distinctly spliced isoforms of its receptor types FGFR-2 and FGFR-3.
Alex Agrotis, Peter Kanellakis, Gina Kostolias, Cheng Wei, Garry Jennings and Alex Bobik.

The proliferation of smooth muscle cells (SMCs) in the intima has been implicated in the development of stenotic occlusive fibrocellular lesions after angioplasty and stenting. We have used a model of balloon catheter injury-induced neointimal development in the rat carotid artery to characterise the expression profile of fibroblast growth factor (FGF) receptors (FGFRs)-2 and -3 and three of their ligands, FGF-1, -2, and –9. Ten days after injury, when neointimal SMC proliferation levels were high, we found distinctly spliced isoforms of FGFR-2 and FGFR-3 in the neointima, and of FGF-9, which possesses unique secretory properties.
The functional significance of this FGFR-2/FGFR-3/FGF-9 co-expression was demonstrated by the ability of recombinant FGF-9 to stimulate neointimal SMC proliferation (4-fold increase in BrdU incorporation) upon infusion at sites of arterial injury, and by the ability of FGF-9 antisense oligonucleotides to significantly inhibit by 50% neointimal SMC proliferation. FGF-9 activity appears to contribute to the neointimal SMC proliferation that occurs after carotid artery injury.

Grants and other funding

Regression of left ventricular hypertrophy in hypertension using gene therapy
Alex Bobik, Ross Hannan and Alex Agrotis, Alfred Research Trust., 2001, $24,500

Commercially funded research activities/clinical trials

NVO-5 and Antioxidant Mechanisms Regulating Intima Development
Novogen Pty Ltd Alex Bobik, 2001, $34,000.

Methods for treating, inhibiting or preventing pathogenic change resulting from vascular injury with an aldosterone antagonist
J Delyani, FR Kenton, J Funder, MR Ward, P Kanellakis and A Bobik, US Patent Application Serial No 09/709,253 (Attorney/Agent: Pharmacia Corporation, Corporate Patent Law Department, PO. Box 5110, Chicago, Illinios 60680-9889

Presentations

Satellite Meeting of the XXIV International Congress of Physiological Sciences Brisbane, Australia (Invited speakers)

Australian Vascular Biology Meeting, Noosa, Australia (Invited speaker)

Clinical Physiology

HEAD
Bronwyn Kingwell
BScHons, PhD (Melbourne)

PROFESSIONAL & TECHNICAL
Melissa Formosa BSc (Victoria)
Brian Drew BScHons (Deakin)

Research Projects

Large artery stiffness as a risk marker and therapeutic target: Structural and genetic aspects.
Tanya Medley, Karen Berry, Kathryn Ferrier, Tamara Waddell, Tony Dart, Melissa Formosa, Brian Drew and Bronwyn Kingwell.

Through a series of integrated human and animal studies, we are investigating how large artery stiffness causes an increase in cardiovascular risk. These studies are directed towards improving cardiovascular risk prediction, confirming that large artery stiffness is both a causative and a reversible risk factor and reducing risk through therapeutic targeting of large artery stiffness.

The hypotheses tested were that:

- identification of the structural and genetic determinants of large artery stiffness would aid in cardiovascular risk prediction.

- large artery stiffening would cause unfavorable haemodynamics and so promote myocardial ischaemia.

- large artery stiffness could be improved through lowering cholesterol levels.

Both large artery stiffening and the elevation in pulse pressure it causes are independent risk factors for cardiovascular mortality. Intrinsically stiff, large vessels may promote atherosclerosis throughout the circulation by elevating pulse pressure which leads to mechanical fatigue and disruption of the endothelium. In addition, elevated aortic stiffness and pulse pressure impair coronary perfusion and increase myocardial work.

Age, atherosclerosis and gender are important factors that influence large artery stiffness, ultimately through their effects on matrix composition which in turn is largely determined by matrix metalloproteinases (MMPs). Genetic variation in matrix proteins and their regulators is an important origin of variation in arterial stiffness. Current work focuses on specific factors thought to be important in modulating large artery stiffness.
Fibrillin-1: The matrix glycoprotein, fibrillin-1, provides both load bearing and anchoring functions within the arterial wall. The fibrillin-1 gene is affected in Marfan syndrome where large artery stiffening is the main cause of aortic dilation.
We have shown that a common genetic variation in fibrillin-1 is associated with stiffer large arteries and more severe coronary artery disease (CAD).

MMPs: As a result of its broad substrate specificity, stromelysin-1 (MMP-3) may be particularly significant to arterial wall remodeling in both aging and disease progression. Our studies have shown that a heterozygosity for a common promoter polymorphism in stromelysin-1 is associated with a reduction in age-related large artery stiffening,

Gender: We have shown that at lease part of the reason why older women have stiffer large arteries than older men is linked to hormonal changes and the menopause.

Coronary artery disease and ischaemic mechanisms:
We have shown that large artery stiffness is related to the severity of CAD and propose that large artery stiffening may promote myocardial ischaemia through elevation of afterload and restriction of coronary perfusion. In support of this idea, we have recently shown that patients with stiffer large vessels have a shorter time to onset of myocardial ischaemia during an exercise test, independently of the severity of CAD, suggesting that aortic stiffness may be a determinant of ischaemic threshold in CAD patients.

Therapeutic targeting of large artery stiffening:
We have recently shown that large artery stiffness is reduced by intensive cholesterol lowering in people with isolated systolic hypertension.

Together these studies identifying specific structural and genetic determinants of large artery stiffness in relation to prediction of cardiovascular risk may permit more appropriate targeting of therapy in relation to age, CAD severity, gender and genotype.

Contraction-mediated glucose uptake as a therapeutic target in type 2 diabetes.
Melissa Formosa, Michael Muhlmann and Glenn McConell (Monash).

The incidence of type 2 diabetes is increasing in Western countries which currently affects 7.6% of adult Australians. Our recent studies yielded the novel result that nitric oxide (NO) is an important mediator of glucose uptake during exercise.

We hypothesised that:

- signaling molecules implicated in the contraction-mediated glucose uptake pathway, including NO, may represent new therapeutic targets for the improvement of resting glycaemic control in patients with type 2 diabetes.

The mechanisms controlling skeletal muscle glucose uptake are of interest because better glucose control means a lower risk of diabetes-related end points. Both insulin action and muscle contraction increase the uptake of glucose into skeletal muscle cells through translocation of the GLUT-4 glucose transporter from the cytosol to the plasma membrane. The two stimuli act synergistically, however the signaling pathways activated by each are different.

Patients with type 2 diabetes have impaired insulin-stimulated GLUT-4 translocation but normal exercise-stimulated GLUT-4 translocation, and their skeletal muscle glucose utilisation during exercise is normal or above normal. These data suggest that people with type 2 diabetes may rely more on contraction-mediated glucose uptake than insulin-stimulated uptake during exercise, in which case signaling molecules activated by contraction might provide the basis for new therapy to stimulate glucose uptake at rest and improve glucose control.

Our group has provided the first evidence for involvement of NO in contraction-mediated glucose uptake in humans. In this study, infusion of a NO synthase (NOS) inhibitor during exercise in young, healthy individuals reduced glucose uptake during exercise by 48%. Extending the study to include people with type 2 diabetes showed that NO-dependent mechanisms accounted for 78% of glucose uptake during exercise compared with healthy individuals. NOS inhibition, which was significantly greater in patients than controls, had no effect on leg blood flow, arterial blood pressure, insulin or glucose concentrations during exercise. This suggested that NO-mediated glucose uptake may compensate for impaired insulin action and account for the normal glucose uptake seen during exercise in people with type 2 diabetes. It may be possible to stimulate the contraction-mediated glucose uptake pathway at rest through the provision of NO donors.

Grants and other funding

Nitric oxide mediated glucose uptake as a potential therapeutic target in type 2 diabetes.
Bronwyn Kingwell, Diabetes Australia, 2001, $35,000

The structural and genetic basis of large artery stiffening.
Bronwyn Kingwell, Rebecca Cooper Foundation, 2001, $12,630

Nitric oxide and type 2 diabetes.
Bronwyn Kingwell, Alfred Hospital Trust, 2001, $25,000

Effects of HRT on arterial stiffness in women with coronary disease.
Bronwyn Kingwell, Australian Menopause Society, 2001, $20,000

Centre of clinical excellence in hospital-based research.
Garry Jennings, Murray Esler, Paul Nestel, Anthony Dart, David Kaye, Jaye Chin-Dusting and Bronwyn Kingwell, National Health and Medical Research Council, 1998-2001, $175,000

Studies on the functional mechanical properties of large conduit arteries and their potential therapeutic impact in cardiovascular disease.
Bronwyn Kingwell, James Cameron and Yean L Lim, National Medical Research Council, Singapore, 2000-2002, $121,627

Presentations

Fourth International Workshop on Structure and Function of Large Arteries, Paris, France (Invited speaker)

10th European Meeting on Hypertension, Milan, Italy

IIIrd Franco-Australian Meeting on Hypertension, Corsica, France

The 2nd Vascular Biology Meeting, Singapore

International Institutes of Health/Baker Symposium, Sydney, Australia (Invited speaker)

Australasian College of Nutrition and Environmental Medicine, Melbourne, Australia (Invited speaker)

Symposium to the XXXIV International Congress of Physiological Sciences, Melbourne, Australia (Invited speaker)

Visiting Scientists

Richard Woodman, University of Western Australia, Australia

Timothy Matthews, University of New Mexico, Albuquerque, New Mexico, USA

Sarah Hamilton, Dartmouth College, USA

Students

PhD
Karen Berry BScHons (Monash)
Scott Bradley BScHons (Monash)
Kathryn North BSc (Monash)
Tanya Medley BSc (Victoria)
Prakash Pillay MBBS

Honours
Anna Ahimastos

Vascular Pharmacology

HEAD
Jaye Chin-Dusting
BScHons, PhD (Monash)

SENIOR SCIENTIFIC
Ruchong Ou MBBS, MD (Kunming, China)
PROFESSIONAL & TECHNICAL
Ann-Maree Jefferis BSc (Melbourne)
Margaret Vincent

Research projects

Angiotensin II stimulated L-arginine uptake by cationic amino acid transporter-1 in human endothelial cells is mediated by angiotensin IV.
Belinda Ahlers, David Kaye & Jaye Chin-Dusting.

The aim of this study was to determine the effect of angiotensin (Ang) II, a potent vasoconstrictor and growth hormone, on the intracellular transport of L-arginine in the human endothelial cell line EA.hy926. L-arginine is transported into EA.hy926 cells by the cationic amino acid transporter-1 (CAT-1). A defective L-arginine - nitric oxide pathway has been implicated in the impaired endothelium dependent vasodilation seen in cardiovascular conditions such as congestive heart failure.

Quiescent EA.hy926 cells were stimulated with Ang II before measurement of [3H] L-arginine transport. The presence of Ang II (10-7 M) increased [3H]L-arginine transport to a maximum at 6 h (28 ± 4 % vs untreated control, $P<0.05$) in the absence of changes in total cellular protein. CAT-1 mRNA levels measured by RNAse protection analysis were maximal by 4h post-stimulation.

Specific inhibition of different Ang receptors showed that the effect seemed to be mediated through Ang IV, an Ang II metabolic degradation product.

Isoflavone metabolites as selective iNOS inhibitors.
Mark Farso, Belinda Ahlers, Ann-Maree-Jefferis, Margaret Vincent & Jaye Chin-Dusting.

The objectives of this study were to investigate the effects on iNOS expression of 18 phytoestrogen metabolites derived from the isoflavones genistein and equol and from the genistein metabolite, daidzein. (The metabolites were kindly made available to us by NOVOGEN Pty Ltd.) In the first series of experiments, the effect of these compounds on LPS/gamma-IFN - activated cytokine-induced nitric oxide production was examined in J774 murine macrophage cells. Nitric oxide production was significantly inhibited with genistein 30 _M ($p<0.05$), but not daidzein, and equimolar-concentrations of the phytoestrogen metabolites Cpd1, Cpd2, Cpd6, Cpd7, Cpd8 and CpdC1 ($p<0.05$).

Dehydroequol (Cpd 1) was found to inhibit the increase in iNOS protein expression in LPS/gamma-IFN-activated J774 cells. The results to date indicate that isoflavone metabolites can reduce the cytokine-induced nitric oxide over-production in murine macrophage cells due to inhibition of iNOS protein expression.

Endothelin-1 Induced peripheral vasodilatation in patients with advanced cirrhosis.
Rhys Vaughan, Peter Angus (Austin Hospital) & Jaye Chin-Dusting.

Patients with advanced cirrhosis have generalised vasodilatation which may contribute to the many complications of the condition, such as ascites, hepato-pulmonary syndrome, hepatorenal syndrome

and fluid retention. This vasodilatation may due in part to an impaired responsiveness to vasopressor hormones such as noradrenaline, angiotensin II and arginine vasopressin, the levels of which correlate with disease severity and prognosis.

Endothelin-1 (ET-1), a 21 amino acid vasoactive peptide is also elevated in patients with cirrhosis. To test the hypothesis that vascular response to ET-1 is altered in cirrhosis and may contribute to the vasodilated state, we measured the in vivo response to ET-1 in male patients with advanced cirrhosis before and after liver transplant, and control subjects.

In response to infused ET-1, the forearm blood flow increased in the cirrhosis group over 60 minutes and decreased in the control group. Heart rate and blood pressure did not change.

The vasodilatation in patients with cirrhosis returned to the normal vasoconstrictor response after liver transplantation, suggesting a possible role of ET-1 in the pathophysiology of the systemic vasodilatation seen in advanced cirrhosis.

Insulin inhibits endothelium-derived relaxing factor in rat isolated mesenteric arteries.
Kimura Masahiko, Jefferis Ann-Maree
& Jaye Chin-Dusting.

The mechanism by which hyperinsulinemia contributes to progression of hypertension and arteriosclerosis is not known. We examined the effects of insulin on endothelium derived relaxing factors.

The effect of insulin on acetylcholine responses was examined in the absence and presence of nitro-L-arginine, indomethacin and high concentrations of KCl (40mM) in segments of rat mesenteric arterioles set up for isometric recordings in myographs. Insulin, nitro-L-arginine, indomethacin or high K+ alone had no effect on responses to acetylcholine. On co-incubation with nitro-L-arginine, but not with indomethacin or high K+, insulin significantly decreased the maximum response to acetylcholine (pre vs post: 84.8 + 8.2% vs 40.7 + 10.2%; $p<0.01$; n=9). Similarly, co-administration of nitro-L-arginine and high K+ significantly decreased acetylcholine responses. Addition of insulin, together with nitro-L-arginine and indomethacin, significantly decreased the maximal response to acetylcholine from 96.6 + 5.3% to 52.9 + 10.8% ($p<0.01$; n=6).

In the system described, attenuation by insulin of acetylcholine responses mediated by endothelium derived relaxing factor was only apparent when nitric oxide was blocked. This action of insulin may play a role in situations where nitric oxide function is decreased, as has been reported to occur in hypertension and arterosclerosis.

Transport of L-arginine into peripheral blood mononuclear cells is not affected by cortisol-induced hypertension in humans.
JPF Chin-Dusting, B Ahlers, JA Whitworth1,
JJ Kelly2, G Jennings & DM Kaye.

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A reduced effect of the L-arginine -nitric oxide system is implicated in cortisol-induced hypertension. The current study investigates whether abnormalities in cellular L-arginine uptake contribute to this phenomenon.

Seven healthy normotensive males were recruited to a double blind, placebo controlled, cross-over study where oral cortisol (50 mg) was taken 6-hourly for 24 hours following a 5-day fixed-salt diet (150 mmol/day). Blood pressure measurements and a full blood examination were obtained before and after each cortisol/placebo treatment period. L-arginine uptake was assessed in peripheral blood mononuclear cells (PBMCs).

Systolic blood pressure increased from 112 + 4 to 119 + 4 mmHg ($p<0.05$). The anticipated changes in blood parameters were also observed with cortisol treatment, including an increase in neutrophils from 3.47 + 0.49 X 109/L to 7.96 + 0.95 X 109/L ($p<0.05$). Cellular L-arginine transport was not affected by active treatment.

We conclude that abnormalities of the L-arginine transport system are not associated with cortisol-induced increases in blood pressure.

Grants and other funding

Centre of clinical excellence in hospital-based research
G Jennings, M Esler, P Nestel, A Dart, D Kaye,
J Chin-Dusting and B Kingwell, National Health and Medical Research Council, 1999-2001, $700,000 over 3 years

Endothelin in hypercholesterolemia
Jaye PF Chin-Dusting and Anthony Dart, Alfred Hospital Research Trusts, 2001, $23,500

Commercially funded research activities/clinical trials

Preclinical and phase 1 studies on the NV-04 program
Jaye Chin-Dusting and Paul Nestel, NOVOGEN Ltd, 2001-2002, $75 000

Vascular effects of PPAR agonist
Jaye Chin-Dusting, Hoffman-La Roche, Basel, Switzerland, 2002, $45,000

Effects of casein A2 on surrogate markers of atherosclerosis
Anthony Dart and Jaye Chin-Dusting,
A2 Productions, Auckland, New Zealand, 2002, $120 000

Presentations

2nd messengers and phosphoproteins meeting, Melbourne

11th European Meeting on Hypertension, Milan, Italy

Students

PhD
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Honours
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H & L Hecht Hormones & the Vasculature

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Research Projects

Effects of endogenous estrogen deficiency on growth and death of aortic smooth muscle cells: studies in aromatase-knockout mice.
Shanhong Ling, Aozhi Dai, Margaret Jones,
Evan Simpson, Krishnakutty Sudhir
and Paul A Komesaroff.

Abnormal growth and death of vascular smooth muscle (VSM) cells are important mechanisms in atherogenesis and plaque progression. We used an estrogen-deficient animal model, the aromatase-knockout (ArKO) mouse, to examine growth and death of VSM cells with and without supplementation with exogenous estrogens.

Primary cultures of VSM cells were established from aortic tissue from 10-11 week-old male and female ArKO and wild type (WT) mice. The absence of aromatase mRNA in cells from ArKO mice was confirmed by RT-PCR and the presence of estrogen receptor was shown by Western blotting. Growth responses to serum or platelet-derived growth factor

(PDGF) BB were significantly lower in ArKO than WT cells. Addition of 17 beta-estradiol (10 nM) significantly improved growth responses to PDGF BB in ArKO cells, but inhibited the response in WT cells. E2 significantly inhibited activity of mitogen activated protein kinase Erk-1 in WT but not in ArKO cells. Apoptosis-related death induced by tumour necrosis factor-alpha (20 ng/mL) was greater in ArKO cells than in WT cells and was attenuated by E2. Similar responses were seen in VSM cells from male and female ArKO.

Testosterone, but not DHEA, enhances apoptosis-related damage in human vascular endothelial cells.
Paul A Komesaroff, Shanhong Ling, Aozhi Dai, Maro RI Williams, Kathy Myles, Rod J Dilley & Krishna Sudhir.

Men and postmenopausal women are at greater risk of cardiovascular disease than premenopausal women. Although estrogens are cardioprotective in women and possibly men, the effects of androgens are unclear. We therefore examined the effects of testosterone (T) and dehydroepiandrosterone (DHEA) on apoptosis, which may be an important mechanism underlying vascular damage, in human vascular endothelial cells (EC) in culture. Male human umbilical vein EC cells (EA.hy926) were grown to confluence in DMEM with 10% serum and apoptosis was induced by serum deprivation for 1-4 days in the presence of T (1-100 nmol/L) or DHEA (1-100 nmol/L). DNA synthesis, DNA fragmentation, cell death, estrogen and androgen receptor density and cell morphology were assessed.

There were 2,374±190 androgen receptors sites/cell and <200 estrogen receptors sites/cell. After 24h serum deprivation, T, but not DHEA, decreased DNA synthesis in a dose-dependent manner. Flutamide (100 nM), an androgen receptor antagonist, abolished this effect of T. The number of severely damaged cells after 48h and 72h was significantly greater with T treatment (13.7±0.47% and 30.2±2.45%, respectively) than with DHEA (10.2±1.13% and 20.6±1.44%) or controls (9.7±1.05% and 23.7±3.0%). After 48h serum deprivation, T significantly increased the number of apoptotic cells and dead cells compared to control. Thus T, but not DHEA, reduced DNA synthesis and enhanced apoptosis in EC following serum deprivation.

Dehydroepiandrosterone improves cardiovascular function via mechanisms independent of androgen and estrogen receptors: in vitro and in vivo studies.
Maro RI Williams, Tye Dawood, Krishna Sudhir & Paul A Komesaroff.

Dehydroepiandrosterone (DHEA) and its pro-hormone DHEA sulphate are the most abundant circulating steroids in humans, and may play a beneficial role in cardiovascular health. It is unknown whether any actions of DHEA in the cardiovascular system are due to direct effects or conversion to other steroids.

We have compared the in vitro effects of DHEA, estradiol (E) and testosterone (T) on (i) the proliferation of endothelial cells (EC) in culture, with and without antagonists of estrogen receptors (ER) and androgen receptors (AR), and (ii) in vivo on indices of endothelial function, arterial compliance and lipids.

For the in vivo studies, 36 healthy postmenopausal women received DHEA (100mg/day) or matching placebo for 3 months. Vascular function was assessed by flow mediated dilation (FMD) of the brachial artery during reactive hyperaemia, and systemic arterial compliance (SAC). DHEA, E and T all enhanced FCS-induced increases in EC proliferation (by 24±2, 17±2 and 28±2% respectively; $p<0.05$). The effects of E and T were blocked by the respective receptor antagonists, which did not affect the action of DHEA.

DHEA increased FMD and SAC but not nitroglycerin-induced vasodilation of the brachial artery. It had no effect on systolic or diastolic blood pressure or mean arterial pressure but significantly reduced total plasma cholesterol (from 6.2 to 5.5 mM; $p<0.05$). These actions of DHEA have potential benefit in the setting of cardiovascular pathophysiology but further studies are required to determine whether they are translated into beneficial clinical endpoints.

The effects of testosterone on glucocorticoid and catecholamine responses to hypoglycaemic audio-visual stress in castrated male sheep.
Tye Dawood, Krishna Sudhir & Paul A Komesaroff.

Stress is an important contributor to disease, including cardiovascular disease. Exposure to stressful stimuli is associated with the activation of the hypothalamic-pituitary-adrenal axis and the release of catecholamines and glucocorticoid hormones. Although it is recognised that estrogens attenuate stress responses, the role of androgens in this setting is uncertain.

We investigated the effects of testosterone on glucocorticoid and catecholamine responses to stressors in castrated rams. Six sheep with testosterone implants and 6 control sheep were psychologically stressed by exposure to a barking dog for 5 minutes. Three weeks later they were exposed to a the physiological stress of hypoglycaemia, induced by injection of Actrapid insulin. ACTH, cortisol, adrenaline and noradrenaline were measured in blood taken at intervals.

Testosterone treatment led to lower baseline glucose levels and also significantly lowered ACTH and cortisol responses to hypoglycaemic stress. Audio-visual stress had no significant effect on ACTH or cortisol responses and catecholamine responses were the same in both groups with either stressor.

Effects of micronised progesterone on cardiovascular risk factors in postmenopausal women.
Suzie Y Honisett, B Pang, Virginia Cable, Catherine VS Black, Krishna Sudhir & Paul A Komesaroff.

There is limited information about the cardiovascular actions of progesterone, despite the potential implications for cardiovascular health in premenopausal women, the concerns about the effects of progestogens in postmenopausal hormonal therapy regimes, and the claims that progesterone alone is of benefit to menopausal women.

We therefore studied the effects of progesterone on cardiovascular risk factors, vascular function and hormone levels in healthy postmenopausal women not taking estrogens. In a randomised, double-blind, cross-over study, the 20 women (mean age of 56.4) were tested before and after 6 weeks of treatment with micronised progesterone (100 mg per day) and matching placebo. We tested plasma sex hormone and lipid concentrations, blood pressure and systemic arterial compliance (SAC) and endothelial function in small and large vessels. Our results showed that in healthy women unprimed by estrogen, micronised progesterone alone had no cardiovascular effects. Any actions of this hormone on the cardiovascular system are likely to occur through its effects on the actions of estrogens.

Physiological levels of estrogen are important for endothelial function in men.
Robert Lew, Krishna Sudhir & Paul Komesaroff.

The many actions of estrogens (E) on the cardiovascular system in women include beneficial affects on lipids and endothelial function. Males produce E via aromatisation of androgenic precursors and there is some evidence that E withdrawal in elderly men leads to potentially deleterious changes and that replacement in hypogonadal men may be beneficial. We therefore examined the effects of aromatase inhibition on endothelial function and lipid levels in healthy young men.

The study followed a placebo controlled, double-blind randomised design. Twenty healthy men, aged 18 to 30 , were randomised to receive either the aromatase inhibitor anastrozole (1mg) or matching placebo. Hormone and lipid levels were measured and endothelial function assessed at baseline and after 6 weeks of treatment. The only significant difference between test groups was that anastrozole led to a decrease in flow mediated dilation (median 6.1±18 to 3.5±18; $p=0.03$). This study showed that a reduction in E levels in healthy young men caused potentially deleterious changes in endothelial function, suggesting that physiological production of E plays a role in cardiovascular health in males.

Rosiglitazone improves cardiovascular risk profiles in postmenopausal women with type II diabetes mellitus.
Suzie Y Honisett, Lily Stojanovska, Krishna Sudhir, Bronwyn A Kingwell & Paul A Komesaroff.

Atherosclerotic vascular disease is the primary cause of mortality in people with type II diabetes. Reduced estrogen levels after menopause may also contribute to the cardiovascular risk profile. The thiazolidinedione, rosiglitazone, is an anti-diabetic agent that binds nuclear peroxisome proliferator activator gamma receptors, which are known to influence lipid and lipoprotein metabolism, glucose homeostasis and vessel wall function. Their effect on estrogen receptors is unknown.

We have investigated whether rosiglitazone reduces cardiovascular risk parameters in postmenopausal women with type II diabetes mellitus. In a double-blind, placebo-controlled study, 18 women were tested before and after 12 weeks of treatment with rosiglitazone. Tests included measurement of metabolic parameters, lipids, blood pressure, flow mediated dilation of the brachial artery (FMD) and systemic arterial compliance (SAC). Rosiglitazone had beneficial effects on all perameters measured, except total plasma cholesterol and cholesterol sub-fractions which were unchanged. We conclude that rosiglitazone improves diabetic control and beneficially affects cardiovascular risk parameters in postmenopausal women with type II diabetes mellitus.

Differential changes in cognition and well-being following dehydroepiandrosterone (DHEA) administration in post-menopausal women.
Tye Dawood, Maro RI Williams, M Waterfall, Krishna Sudhir & Paul A Komesaroff.

The current study sought to examine changes in cognition and well-being, including anxiety and depression, following DHEA supplementation in healthy post-menopausal women not taking estrogen. In a double-blind, placebo-controlled parallel arm trial, participants received either oral DHEA (100mg daily) or placebo for three months. Cognition was assessed by the Wechsler Memory Scale-Revised (WMS-R), general well-being was assessed using the General Health Questionnaire (GHQ-60), and anxiety and depression were evaluated using the Hospital Anxiety and Depression Scale (HADS) 1994.

Thirteen of sixteen women showed improvements after DHEA in memory and numeric cognition. No other changes were recorded. We conclude that DHEA administration in healthy post-menopausal women not on hormone replacement is beneficial for memory and certain cognitive tasks.

Effects of estrogens and anti-estrogens on growth of human desmoid tumour cells.
Aozhi Dai, Shanhong Ling, Joanna E Paddle-Ledinek, Krishnakutty Sudhir, Jonathan W Serpell & Paul A Komesaroff.

Desmoid tumours are rare fibroblastic tumours of mesenchymal origin that occur sporadically - mainly in healthy young women - and as part of the familial cancer syndrome, Gardner's Syndrome. Typically, they are fast growing, resistant to therapy and have a high local recurrence rate after surgery. Recent evidence has shown that in some cases, despite the apparent lack of estrogen receptors (ER), the ER partial antagonist, tamoxifen, may prolong the response to therapy.

We have examined growth responses to 17beta-estradiol (E2) and the ER antagonists tamoxifen and ICI 182,780 (ICI) in vitro in cultured desmoid tumour cell lines from patients. Western blotting showed that expression of ER-alpha was relatively high in the cells of female origin and low or absent in those from males. Expression of both ER-beta and the androgen receptor was low in female and relatively high in male cells. Growth of cells derived from a female patient with familial adenomatous polyposis and an anterior rectus sheath tumour sensitive to tamoxifen treatment responded vigorously to E2 treatment in a dose-dependent way. In cells derived from two other patients with the sporadic form of the disease, 100 nM E2 caused a 7-9% increase in growth. A fourth cell line – from a male patient – showed no response to E2. In those cells stimulated by estrogen, both tamoxifen and ICI completely inhibited E2-induced proliferation.

We conclude that desmoid tumours display varying phenotypes. In those forms expressing ER-alpha, estrogens may stimulate tumour growth and anti-estrogens may block this effect.

A randomised, double blind, placebo-controlled study of the effects of acupuncture on menopausal symptoms.
Paul A Komesaroff, Euhana Varigos, Virginia Cable & Krishna Sudhir.

We conducted a study of the effects of laser acupuncture in the treatment of menopausal symptoms using a device that allowed both operator and patient to be blinded as to whether it was active or inactive.
The study followed a randomised, double blinded, placebo-controlled, parallel arm design. Forty healthy women with troublesome menopausal symptoms were randomised to receive active acupuncture or placebo. Treatment was carried about fortnightly for an average of 20 weeks. Symptoms were assessed on a weekly basis using a diary to record the number of diurnal and nocturnal flushes and the non-flushing symptom score.

At the end of the treatment period, weekly scores had significantly declined in all three symptom categories in both the active and the placebo groups. There were no significant differences between the placebo and active groups.

This results of this study suggest that the use of laser acupuncture appears to have little effect on menopausal symptoms.

Students

PhD
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Robert Lew MBBS (Melbourne) FRACP
Suzy Honisett BScHons (Victoria)

MSc
Tye Dawood, BSc (Monash)
Honours
Kelvin Lam

Morphology

HEAD
Rodney Dilley
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PROFESSIONAL & TECHNICAL
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Rosemary van Driel BSc

Research Projects

Role of plasminogen activator inhibitor-1 (PAI-1) in cardiovascular remodelling mechanisms: Chronic angiotensin II infusion in mice.
Rodney Dilley, Rosemary van Driel, Natalie Kvalheim, David Loskutoff* & Stefan Koschnick*
(*The Scripps Research Institute).

The walls of the blood vessels and heart become thicker and stiffer in response to increases blood pressure. These processes may contribute to worsening of the hypertension and promotion of heart disease. We have continued to examine the development of fibrosis and remodelling, and how they are regulated, in mice made hypertensive by chronic infusion of angiotensin II.

We looked at the fibrinolytic system which removes blood clots and controls fibrous tissue components during remodeling by assessing the expression of PAI-1, tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA) and vitronectin.
We found that this system is activated in the heart and aorta of mice made hypertensive with angiotensin infusion, with increases in PAI-1 and tPA mRNA in the vessel wall and heart during fibrosis. There was no change in uPA or vitronectin mRNA. Regulation of these systems by antihypertensive drug treatment, specifically those which regulate angiotensin II levels, may be able to reduce the fibrosis and stiffening which develop in hypertension.

Regulation of vascular smooth muscle cell proliferation by heparin and related oligosaccharides.
Rodney Dilley & Peter Little.

It is widely held that heparin is able to reduce proliferation and migration of smooth muscle cells – the cells from blood vessel walls that contribute most to artery disease. Heparin is large, and its high anticoagulant activity make it an unsuitable candidate for chronic drug treatment. We have tested a range of smaller molecules derived from heparin in a cell culture system and found them to have similar anti-growth activity to heparin without the anticoagulant side effects. We are now testing these heparin derivatives in more complex animal systems for their potential as antigrowth drugs in blood vessel walls.

Hypertrophy in diabetic mesenteric artery but not basilar artery is reflected in changes to Na/H exchange.
Rodney Dilley & Peter Little.

The arteries supplying the intestines have been shown to have thicker walls in rats with diabetes, partly through increased activity of the system that

regulates cell acidity. As peoples with diabetes are at very high risk of vascular disease, we tested the proposal that all blood vessels of diabetics may be similarly affected, perhaps as a result of the increased blood glucose. We found no changes in the acidity and growth of cells in the basilar arteries that supply the brain and are similar in size to mesenteric arteries, suggesting that the mesenteric changes were unlikely to be due to direct vascular effects of high glucose.

Presentations

International Society for Heart Research, Winnipeg, Canada;

Australian Vascular Biology Society, Noosa, Australia

High Blood Pressure Research Council, Melbourne, Australia

Vascular Diabetes Laboratory

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PROFESSIONAL & TECHNICAL
Melanie Ivey (RMIT)

Research projects

Actions of PPAR-gamma ligands on proliferation, migration and proteolgycan production in human vascular smooth muscle cells.
Stephanie T de Dios, Garry L Jennings & Peter J Little.

The aim of this study was to determine if the PPAR-gamma ligands, which are the latest class of drugs for the treatment of hyperglycemia in type 2 diabetes, have direct actions on vascular smooth muscle cells. Diabetes is known to hasten the onset of cardiovascular disease.

The PPAR-gamma ligands, troglitazone, rosiglitazone and pioglitazone, are insulin sensitisers which are known to improve the body's response to insulin and hence lead to lower blood glucose levels. These ligands may also have direct actions on blood vessels. We are looking at smooth muscle cells isolated from the three types of vessels used in coronary artery by-pass grafting as these will be important in patients with diabetes who undergo by-pass surgery. The vessels are the internal mammary artery, the radial artery and the saphenous vein.

We found that the three PPAR-gamma ligands tested caused inhibition of proliferation for each of the human vascular smooth muscle cell types, which is likely to be a favorable response in reducing vascular disease. The compounds also inhibited proteoglycan production with potency troglitazone>rosiglitazone=pioglitazone.

Glitazones have favorable actions on vascular smooth muscle which may slow the development of vascular disease in people with diabetes.

Role of PPAR-alpha ligands ("fibrates") in modification of proteoglycan biosynthesis and lipoprotein binding.
Julie Nigro, Garry L Jennings & Peter J Little.

This study was performed to determine if the fibrate drugs used to treat hyper-triglyceridemia have direct actions on vascular smooth muscle to prevent vascular disease.

People with elevated blood triglyceride levels, including those with diabetes, are often treated with a family of drugs known as fibrates which have recently been recognized as PPAR-alpha ligands. Evidence suggests that such drugs may have actions on blood vessels which reduce the impact of the vascular disease caused, in part, by elevated blood lipids.. We are investigating the role of fibrates in reducing the size of the sugar chains on proteoglycans – large negatively charged molecules – and thereby reducing their ability to bind lipoproteins.

Human vascular smooth muscle cells from surgical tissue were used in these studies. The fibrates gemfibrozil (available in Australia) and fenofibrate (used in the USA) both inhibited the production of proteoglycans and reduced the size of the sugar chains which bind lipoproteins.

Fibrates have actions on human vascular smooth muscle cells which may complement their primary action of lowering triglyceride levels.

Direct actions of Advanced Glycation End Products (AGEs) on human vascular smooth muscle.
Mandy Ballinger, Rodney J Dilley & Peter J Little.

AGEs accelerate the development of atherosclerosis in people with diabetes. This study aimed to test whether they had this effect through direct effects on vascular smooth muscle. Elevated blood glucose levels in people with diabetes lead to the accelerated formation of AGEs which are glucose conjugates with a wide variety of proteins. They are thought to have wide-spread deleterious effects on the cardiovascular system. Agents that inhibit the formation of AGEs have a dramatic effect on reducing atherosclerosis in animal models of disease.

We prepared a spectrum of AGEs and showed that human vascular smooth muscle cells have receptors for AGEs. However, we found no evidence that the glycated proteins affected any vascular smooth muscle cell responses, including accelerated metabolism, new protein biosynthesis and the formation of proteoglycans.

The strong association between AGEs and the presence of atherosclerois might arise from actions on other cells such as macrophages in the area associated with atherosclerotic plaques.

Regulation of glucose metabolism and ATP levels in human vascular smooth muscle.
Melanie Ivey, Gillian Seaton, Julie Nigro, Stephanie de Dios, Rodney Dilley & Peter J Little.

Diabetes is associated with accelerated vascular disease, assumed to result from the direct interaction of the high glucose concentrations with the blood vessels. However, there have been no consistent demonstrations of the effects of glucose on isolated vascular smooth muscle cells, due possibly to a lack of knowledge of glucose metabolism and its regulation in these cells. The aim of this study was to describe the regulation of glucose metabolism by vascular smooth muscle cells.

We have shown that the rate of glucose metabolism in vascular smooth muscle cells is extremely high over a range of glucose concentrations, which could potentially lead to a limitation in the availablity of ATP. Glucose metabolism was modified by growth factors and drugs.

Enhanced understanding of the factors influencing glucose metabolism in vascular smooth muscle cells may allow a greater understanding of the role of glucose in causing accelerated vascular disease in people with diabetes.

Grants and other funding

The study of the vascular actions of the new PPAR-g agent, pioglitazone
Peter Little, Eli Lilly and Co Endocrinology Research Grant, 2001, $20,000

Visiting scientists

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Presentations

American Heart Association, Philadelphia, USA

Australian Diabetes Society Annual Scientific Meeting, Gold Coast, Australia

Australia Vascular Biology Society 9th Annual Scientific Meeting, Noosa, Australia
Satellite meeting of the International Union of Physiological Societies – Diabetes complications, Melbourne, Australia

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PROFESSIONAL & TECHNICAL
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Research projects

Structure-function studies of apoA-I variants; site-directed mutagenesis and natural mutations.
Dmitri Sviridov and Anh Hoang.

Five mutants of apolipoprotein A-I, apoA-I(_63-73), apoA-I(_140-150), apoA-I(63-73@40-150), apoA-I(R149V), apoA-I(P143A) were compared with human plasma apoA-I for their ability to promote cholesterol and phospholipid efflux from HepG2 cells. Lipid-free apoA-I(_63-73) had a significantly lower capacity to promote cholesterol and phospholipid efflux, while mutations apoA-I(_140-150) and apoA-I(P143A) affected phospholipid efflux only. When the apoA-I mutants were provided as POPC lipid complexes, apoA-I(63-73@140-150) and apoA-I(_140-150) affected cholesterol efflux.

Five natural mutations of apoA-I; apoA-I(A95D), apoA-I(Y100H), apoA-I(E110K), apoA-I(V156E) and apoA-I(H162Q) were studied for their ability to bind lipids, activate lecithin-cholesterol acyltransferase (LCAT) and promote cholesterol efflux. Mutants apoA-I(E110K), apoA-I(V156E) and apoA-I(H162Q) caused less activation of LCAT than natural apoA-1.

The following conclusions could be made from the combined data: i) the central of apoA-I region, between amino acids 100-160, is likely to be the site of interaction with LCAT; ii) regions 210-243 and 63-100 are the lipid-binding sites of apoA-I and are also required for the efflux of lipids to lipid-free apoA-I, suggesting that initial lipidation of apoA-I is rate-limiting in efflux; iii) for cholesterol efflux from cells to lipidated apoA-I, the central region is important in addition to the lipid-binding regions, suggesting possible involvement of the central region in the interaction of apoA-1 with cells.

Delineation of the role of pre-beta1-HDL in cholesterol efflux using isolated pre-beta1-HDL.
Dmitri Sviridov, Kally Theodore, Anh Hoang & Paul Nestel.

The role of the pre-beta1 form of HDL in cholesterol efflux was investigated by isolating it from human plasma using a monoclonal antibody specific for pre-beta1-HDL. Compared with whole plasma, pre-beta1-HDL-deficient plasma was equally efficient in promoting cholesterol efflux from human skin fibroblasts and THP-1 human macrophage cells. When added at the same apoA-I concentration, pre-beta1-HDL was less effective than whole plasma in promoting cholesterol efflux from fibroblasts but equally effective with THP-1 cells. However, pre-beta1-HDL-deficient plasma reconstituted with 16% pre-beta1-HDL was more active than whole plasma, demonstrating that pre-beta1-HDL promotes cholesterol efflux.

The amount of cellular cholesterol present in re-isolated pre-beta1-HDL was 1.5- to 2- fold greater after incubation of cells with whole plasma than it was in pre-beta1-HDL-deficient plasma or plasma treated with the anti pre-beta1-HDL antibody. However, anti pre-beta1-HDL antibody did not inhibit cholesterol efflux. We conclude that pre-beta1-HDL are capable of taking up cellular cholesterol, but their presence in plasma is not essential for cholesterol efflux, at least in vitro. Instead, pre-beta1-HDL may be the first product of apoA-I lipidation during formation of HDL rather than having a major role in transferring cellular cholesterol to HDL.

Protein engineering to produce an antibody to ABCA1.
Dmitri Sviridov & Ying Fu.

Loss of ABCA1 function through mutation causes Tangier disease, a disorder characterised by the absence of HDL and reverse cholesterol transport. In the absence of ABCA1, cells are unable to transfer lipids to lipid-free apoA-I to form HDL. Although ABCA1 is a key element in the formation of HDL, its role in overall cholesterol efflux from cells is unclear. Studies in this area are hampered by the lack of an effective antibody, because ABCA1 is similar between species and therefore not immunogenic. By linking parts of ABCA1 with other proteins and peptides we have obtained active polyclonal antibody.

Grants and other funding

Delineation of the role of apolipoprotein A-I in the transport of cholesterol from intracellular compartments to the caveolae
D Sviridov, National Heart Foundation of Australia, 2001, $50,000

Delineation of the role of prebeta1-HDL in cholesterol efflux
D Sviridov and P Nestel, Daiichi Pure Chemicals, 2001, $70,000

Visiting scientists

Gabrielle Gallon PhD (Toulouse)

Presentations

2nd Annual Conference on Arteriosclerosis, Thrombosis and Vascular Biology, Washington DC, USA

Cardiovascular Nutrition

HEAD
Professor Paul Nestel AO
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Sylvia Pomeroy BSc, MPH (Monash)
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Research projects

Effects of red clover isoflavones on arterial functions and plasma lipids.
The hypotheses for this study were three-fold: that isoflavones would lower cardiovascular risk through beneficial effects on plasma lipids and arterial function; that two major isoflavones – biochanin B (which is rapidly converted to genistein) and formononetin F (daidzein) – would have different effects; and that there would be a gender effect. Eighty middle-aged male and female subjects received either B or F in a randomised, double-blind placebo-controlled trial.

The results showed that only biochanin B lowered plasma low density lipoprotein cholesterol significantly, and then, only in men. Importantly, the trial also showed that pure isoflavones at a dose of 40 mg daily significantly improved central pulse wave velocity (a measure of arterial elasticity) and flow mediated dilatation.

(The study was carried out with Dr Helena Teede and Prof Barry McGrath from Monash).

Cholesterol-lowering efficacy of plant sterols within novel margarines and low-fat foods.
Two separate studies were conducted, the first to test the efficacy of sterol esters delivered in foods other than the conventional spreads and the second to examine the safety of high doses of sterols such as might occur if there were widespread consumption in multiple foods. In a randomised, controlled, cross-over design trial, we discovered that food matrix did not significantly alter the potency of the sterols, although some foods were superior to others at delivering the phytosterols.

The second study is confidential to the sponsoring company and to the food regulators who requested the information. It was carried out jointly with Dr Peter Clifton (CSIRO, Adelaide) and Dr David Sullivan.

Comparison of absorption of isoflavones as aglycones or glycosides.
This study was a randomised, blinded, placebo-controlled, cross-over trial of different mixtures of isoflavones similar to those currently used to fortify foods. In natural, isoflavones are conjugated as glycosides that are cleaved by bowel micro-organisms

before their absorption as aglycones. In theory, the absorption and excretion of the two forms, and their metabolism, might differ. However, we found similar absorption and excretion, irrespective of the nature or the conjugation status of several isoflavones.

Potential cardiovascular benefits of chickpeas.
The nutritional value of legumes less commonly consumed by Western populations, such as chickpeas, has been generally under-researched in terms of chronic cardiovascular disease prevention, although Indian and Middle-Eastern studies have reported benefits such as cholesterol-lowering.

We compared, in 22 middle-aged subjects, the effects of substantial daily consumption of chickpea-containing foods with similar wheat-based foods that had comparable macronutrient and fibre composition. Two diets were designed, each with a view to conferring cardiovascular benefit. They differed only in the micronutrients present in chickpeas.
We found no benefit unique to chickpeas in any of the following: plasma lipid levels, glucose and insulin responses to glucose, blood pressure and augmentation index, and circulating levels of oxidised low density lipoproteins.

Metabolism of the pre beta-1 subfraction of High Density Lipoprotein (HDL).
(With Dmitri Sviridov)
We have access to a non-commercial assay for pre beta-1 HDL, the putative initial acceptor of cellular cholesterol. The major studies related to defining the role of this HDL subfraction in cholesterol efflux (in vitro studies) and as a potential diagnostic marker of the efficiency of cholesterol transport in subjects with high and low concentrations of HDL (in vivo studies).

Grants and other funding

Sponsored industry grants:

Effects of red clover isoflavones on arterial functions and plasma lipids

Comparison of absorption of isoflavones as aglycones or glycosides
Paul Nestel, Novogen Ltd

Cholesterol-lowering efficacy of plant sterols within novel margarines and low-fat foods.
Paul Nestel, Goodman Fielder

Potential cardiovascular benefits of chickpeas.
Paul Nestel, Grains Research & Development Corporation

Metabolism of the pre beta-1 subfraction of High Density Lipoprotein (HDL)
Paul Nestel, Daiichi Chemical Company
Amounts of grants are confidential but known to BMRI

Visiting scientists

Dr Nobuyo Tsunoda PhD, Josai University, Japan.

Presentations

Future Forum (Cardiovascular Conference), London, UK

ILSI-CSIRO Conference on Biomarkers as Surrogates for Health Outcomes, Adelaide, Australia (invited lecture)

Conference on Health Benefits of Omega-3 Fatty Acids, Wollongong, Australia (invited lecture)

Conference on Health Aspects of Phytosterols, Stresa, Italy (invited lecture)

Korean Atherosclerosis Society Scientific Meeting, Seoul, Korea (invited lecture)

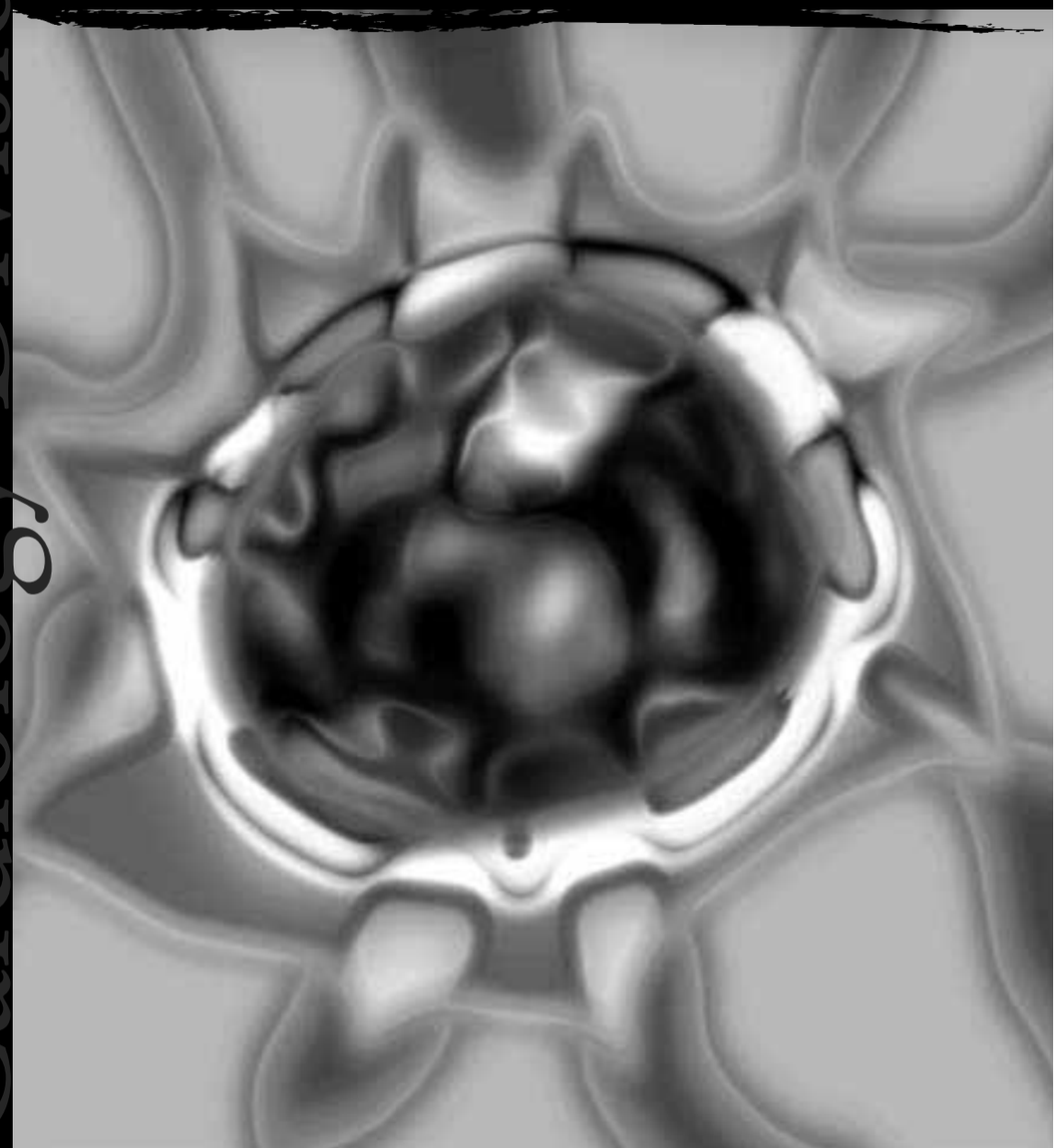
Drugs Affecting Lipid Metabolism, New York, USA

Conference on Functional Foods, Paris, France (invited lecture)

American Heart Association, Anaheim, USA

Conference on Heart Protection Study, Singapore

Australian Nutrition Society Scientific meeting, Canberra, Australia



Molecular Cardiology and Signalling Group

Cellular Biochemistry

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Research projects

Phospholipase C isoforms mediating receptor responses in cardiomyocytes.
Jane Arthur, Scot Matkovich, Bronwyn Kenny & Elizabeth Woodcock.

The project aimed to identify the different isoforms of phospholipase C (PLC) that mediate inositol phosphate responses to alpha1-adrenergc receptors, P2Y2-purinergic receptors and raised Ca2+ in neonatal cardiomyocytes.

We used adenoviruses to over-express the different PLC isoforms in cardiomyocytes. In addition, we investigated selective phosphorylation of PLC isoforms following receptor activation.

We demonstrated that PLC beta1 selectively mediates responses to alpha1-receptor stimulation, while PLC beta3 couples selectively to purinergic receptors. Calcium responses were mediated by both PLC beta1 and PLC beta3 but not by PLC delta1, the most sensitive isoform in vitro.

We conclude that the specificity of PLC activation is determined by the receptor, as both alpha1-receptors and P2Y2-receptors activate the same G protein, Gq.

Hypertrophic signaling from P2Y2-purinergic receptors.
James Morris, Tam Pham, Bronwyn Kenny & Elizabeth Woodcock.

The project aimed to establish whether activation of Gq-coupled P2Y2-receptors stimulated hypertrophic signaling pathways.

We transfected cardiomyocytes with genes comprising atrial natriuretic peptide (ANP) or myosin light chain (MLC) promoters attached to luciferase genes to measure promoter activity, as an end point in hypertrophic signaling. We directly measured phosphorylation of the various MAP kinases and examined tyrosine phosphorylation of certain intermediates in response to receptor activation.

We found that the purinergic agonist ATP activated early responses, such as MAP kinase activation, but was ineffective in stimulating ANP and MLC responses. ATP was found to be inhibitory to ANP and MLC activation mediated by other receptors. This suggested that ATP had multiple effects on growth signaling, acutely stimulating responses but acting as an inhibitor with prolonged stimulation. To investigate this further, we used UTP as an alternative agonist at P2Y2-receptors. Unlike ATP, UTP stimulated MLC expression, providing evidence that P2Y2-receptors are stimulatory to cardiomyocyte growth pathways. UTP but not ATP also stimulated increases in cardiomyocyte size.

Inositol polyphosphate 1'phosphatase as an anti-hypertrophic effector.
Elizabeth Woodcock, Jane Arthur & Ross Hannan.

The project examined whether inositol polyphosphate 1'phosphatase (INPP) inhibited cardiomyocyte growth signaling.

We cloned INPP from a human heart cDNA library and used the gene in cardiomyocyte co-transfection experiments. Effects on inositol phosphate signaling were examined using anion-exchange HPLC. GFP-tagged constructs were used to determine effects of INPP on cell morphology.

We showed that INPP inhibited hypertrophic signaling without causing general damage or cell death. We conclude that the substrate of INPP, inositol(1,4) bisphosphate stimulates hypertrophic signaling.

Visiting scientists

Joan Heller Brown, University of California, San Diego, USA

Presentations

Molecular Pharmacology Gordon Research Conference, Ventura, California, USA

11th International conference on Second Messengers and Phosphoproteins, Melbourne, Australia

Students

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Gene Transcription

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Research projects

Mechanisms regulating ribosomal gene transcription during cardiac hypertrophy.
Ross Hannan and Yves Brandenburger.

The focus of our laboratory is to understand the regulation of transcription of the mammalian ribosomal genes (rDNA) by RNA polymerase I. Transcription of rDNA is the rate-limiting step in the synthesis of functional ribosomes (ribogenesis) and is therefore likely to influence cellular growth, both in normal development and disease states.

We have identified a ribosomal gene transcription factor, UBF, which promotes ribogenesis during hypertrophic growth of cardiomyocytes in culture and proliferative growth of transformed fibroblasts. We are continuing to study the role of UBF in the regulation of rDNA transcription and cellular growth. Site-directed mutagenesis is being used to identify the domains and regulatory phospho-residues in the UBF protein that account for its altered activity during cardiac growth. To determine whether inhibition of the UBF gene can cause regression if not prevention of hypertrophic heart disease, we are using gene therapy based on adenoviruses and mouse transgenics to introduce dominant negative versions of UBF into the hearts of mice.

Regulation of ribosomal gene (rDNA) transcription by p70/p85S6K.
Ross Hannan, Yves Brandenburger, R. Pearson (Peter MacCallum Institute) & Kate Hannan (PMCI).

p70/p85S6K (S6K1) is known to be essential in the regulation of growth and proliferation. The effects of S6K have been attributed to phosphorylation of S6 ribosomal protein. Phosphorylated S5 stimulates translation of mRNA containing a 5' oligopyrimidine tract (5'TOP) including mRNAs encoding ribosomal proteins and certain translation regulatory factors.

We have begun to investigate how S6K1 regulates growth in terminally differentiated cardiomyocytes and proliferating fibroblasts. We have shown for the first time that S6K1 regulates rDNA transcription, independently of its effect on 5'TOP mRNA, by phosphorylating additional nuclear substrates.
We are currently using proteomic and classic biochemical approaches to identify the nuclear target of S6K1. In addition, we are using mouse embryonic fibroblast isolated from S6K2 null mice to determine if S6K2, a novel homolog of S6K1, also contributes to the regulation of cardiac hypertrophy and cell growth.

EGF receptor transactivation in GPCR-mediated cardiac hypertrophy.
Walter Thomas, Hongwei Qian, Ross Hannan & Yves Brandenburger.

The initiation of left ventricular hypertrophy (LVH) in essential hypertension, aortic stenosis and myocardial infarction correlates with altered expression and activity of G protein-coupled receptors (GPCRs), particularly the angiotensin type 1 receptor (AT1R). Traditionally, the contribution of GPCRs to LVH has been considered in terms of classical G protein-mediated intracellular signals emanating from these receptors (typically, Galphaq-induced Ca2+ or protein kinase C).

Using adenoviral approaches, we have unequivocally demonstrated that AngII promotes cardiac myocyte growth via AT1A receptors and have shown that the growth-dependent effects of AngII are mediated via AT1R-dependent transactivation of the EGF receptor and subsequent MAPK signalling. Current studies are focused on understanding the process of EGFR trans-activation from a molecular, cellular and in vivo perspective.

Regulation of rDNA transcription by Myc/Mad1.
Ross Hannan & G. McArthur (PMCI).

Hypertrophy of adult myocytes, whether in vivo or in vitro, is associated with re-induction of genes that are normally expressed only in the fetal heart. The proto-oncogene c-myc was one of the first genes identified as part of this inducible gene program. However, until recently, the contribution of c-myc to the development of cardiac hypertrophy was unknown. Studies using inducible transgenesis have demonstrated over expression of c-myc in the heart leads to cardiac hypertrophy.

We have examined the molecular mechanism by which c-myc and Mad-1, an inhibitor of myc function, regulate cardiac cellular growth. Using a combination of cell lines that over express c-myc and Mad-1(-/-) mice, we have shown that Myc and Mad-1 regulate ribosome biogenesis, which is partly how they effect changes in cellular growth. We are currently using gene targeting and over-expression studies to determine the molecular mechanism by which c-myc/Mad-1 affects the synthesis of ribosomes.

Grants and other funding

Mechanisms regulating ribosomal gene transcription during cardiac hypertrophy
Ross Hannan, National Health and Medical Research Council, 2001-2005

Role of urotensin II, a novel vasoconstrictor factor in cardiovascular disease
Henry Krum and Ross Hannan, National Health and Medical Research Council, 2001-2003

Presentations

23rd Annual Lorne Genome Conference, Lorne, Australia

17th Wilhelm Bernard's Workshop for the Nucleus, Arcachon, France

High Blood Pressure Research Council Meeting, Melbourne Australia

Molecular Physiology

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Ross Hannan BScHons, PhD (Tasmania)
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Kathy Myles DipAppSc (RMIT), BScHons (Melbourne)
Rebecca Ridings DipLabTech (Peninsula TAFE)

Research projects

Cardiac corticosteroid receptors and 11beta-hydroxysteroid dehydrogenase isoforms.
Karen Sheppard & Dominic Autelitano.

This project aimed to determine the expression of corticosteroid receptors and 11betaHSD isoforms in cardiac myocytes and fibroblasts and also the role of 11betaHSD isoforms in modulating the glucocorticoid response.

The studies a number of laboratory techniques, including tissue culture, RNase protection assays, receptor binding assays and steroid metabolism assays.

We showed that there was differential expression of glucocorticoid receptor and mineralocorticoid receptor in cardiac myocytes and fibroblasts and that only 11betaHSD1 was present in these cells. The study also demonstrated that 11betaHSD1 transforms 11-dehydrocorticosterone into transcriptionally active glucocorticoid in both cardiac myocytes and fibroblasts.

These data predict that the mineralocorticoid receptor in cardiac myocytes would mediate glucocorticoid effects, and, due to the absence of mineralocorticoid receptor in cardiac fibroblasts, mineralocorticoid-induced fibrosis is unlikely to be a direct effect of mineralocorticoids on these cells. In addition, because heart can utilise both circulating corticosterone and 11-dehydro-corticosterone as a source of glucocorticoid, it is constantly exposed to high levels of endogenous glucocorticoid, suggesting an important homeostatic role of these steroids on heart.

Role of Sgk in cardiac function.
Karen Sheppard & Dominic Autelitano.

The aims of this project were to determine the potential role of Serum- and Glucocorticoid-induced Kinase (SGK) in cardiac physiology and pathophysiology, and to explore both the interaction between corticosteroid signalling and protein kinase signalling and the potential impact on cardiac hypertrophy and fibrosis of stimulating both of these pathways.

The methods used included tissue culture, RT-PCR, RNase protection assay, transfection/reporter assays and Western blots.

Glucocorticoids, mineralocorticoids and the inactive glucocorticoid metabolite, 11-dehydro-corticosterone, caused transcriptional induction of SGK in both cardiac myocytes and fibroblasts. Some agents which induce cardiomyocyte hypertrophy potentiate this corticosteroid-induced SGK gene transcription, suggesting that in cardiac cells there is cross-talk between corticosteroid signalling and kinase signalling pathways.

Peptide Biology

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Mike Yarski PhD (California)
PROFESSIONAL & TECHNICAL
Shane Reeve
Cath Hamilton

Research projects

The major aim of our research program is to better understand the role played by vasoactive peptides in the regulation of cardiovascular function. We are especially interested in the peptidases that generate and metabolise peptide signals, with a view to designing and characterising specific peptidase inhibitors, which may be of therapeutic value.

Development and Characterisation of Novel Potent and Stable Inhibitors of Endopeptidase EC 3.4.24.15.
Corie N Shrimpton, Giovanni Abbenante, Rebecca A Lew, C Hamilton & A Ian Smith.

Solid phase synthesis was used to prepare a series of modifications to the selective and potent inhibitor of endopeptidase EC 3.4.24.15 (EP24.15), N-[1(R,S)-carboxy-3-phenylpropyl]-Ala-Ala-Tyr-p-aminobenzoate (cFP), which is degraded at the Ala-Tyr bond, thus severely limiting its utility in vivo. Reducing the amide bond between the Ala and Tyr decreased the potency of the inhibitor by 1000 fold. However, the replacement of the second alanine immediately adjacent to the tyrosine with alpha-aminoisobutyric acid gave a compound (JA-2) equally potent to cFP with a Ki of 23 nM. Like cFP, JA-2 inhibited the closely related endopeptidase EP24.16, 20-30 fold less potently than its inhibition of endopeptidase EP24.15. JA-2 did not inhibit the other thermolysin-like endopeptidases ACE, ECE and NEP.

To test its biological stability, JA-2 was incubated with a number of ovine tissue extracts, both membraneous and soluble. JA-2 remained intact after 48 hours of incubation with all tissues examined, in contrast to cFP. Further modifications to the JA-2 compound failed to improve its inhibitory potency. Hence JA-2 is a potent, biologically stable inhibitor of EP24.15 and as such, provides a valuable tool for further assessing the biological functions of this endopeptidase.

The Design and Analysis of Novel Metalloendopeptidase Inhibitors: Applications for Blood Pressure Regulation and Blood Brain Barrier Permeability.
U M Norman, R A Lew, M Hickey, R Evans, C Shrimpton & A Ian Smith.

We have developed a novel inhibitor of the metalloendopeptidases EC 3.4.24.15 (EP24.15) and EC 3.4.24.16 (EP24.16) called N-[1-(R,S)-carboxy-3-phenylpropyl]-Ala-Aib-Tyr-p-aminobenzoate (JA-2), in which alpha-aminoisobutyric acid is substituted for an alanine in another well-described, but unstable, inhibitor, cFP-AAY-pAB. This substitution increases the resistance of the inhibitor to degradation without altering potency.

We investigated the effects of JA-2 on the responses of mean arterial pressure to bradykinin, angiotensin I and angiotensin II in conscious rabbits.
The depressor responses to both low and high doses of bradykinin were increased in the 30 min after JA-2 administration The hypertensive effects of angiotensins I and II were unaltered, indicating that the bradykinin-potentiating effects were not due to ACE inhibition. Bradykinin potentiation was undiminished four hours after JA-2 injection.

We then examined the role these enzymes might play in regulating blood brain barrier (BBB) permeability. BBB permeability was assessed using a rat model by determining the leakage of FITC-labelled molecules out of the Pial (brain surface) vasculature via an acute cranial window preparation. Superfusion of the brain surface with bradykinin induced a rapid and reversible increase in leakage of FITC labelled dextrans. Administration of JA-2 decreased this response unless the bradykinin B2 receptor antagonist (HOE 140) was given beforehand.

Role of Endothelial Cell Endopeptidases EC 3.4.24.15 and EC 3.4.24.16 in Bradykinin Metabolism.
Rebecca A Lew*, ShaneReeve, Peter J Little, Vincent Dive & A Ian Smith.

Bradykinin (BK) is efficiently cleaved in vitro at the Phe5-Ser6 bond by the closely-related metallo-endopeptidases EC 3.4.24.15 (EP24.15) and 24.16 (EP24.16); however, the role of these enzymes in BK metabolism within the vasculature is unknown.
In this study, we have characterized EP24.15 and EP24.16 in cultured endothelial cells derived from the sheep aorta. Bradykinin was readily degraded to BK1-5 by the soluble (cytosolic) fraction of endothelial cells (rate = 160 nmoles/mg protein/hr); degradation was completely inhibited by EDTA or an EP24.15/16 inhibitor.

Inhibitors of angiotensin-converting enzyme, neutral endopeptidase and prolyl endopeptidase were ineffective. Bradykinin was also degraded by endothelial cell membranes (rate = 14 nmoles/mg protein/hr), producing BK1-7 (due to neutral endopeptidase) and BK1-5 (due to EP24.15/16). Both secreted and cell-associated EP24.15/16-like activity was detected when BK was incubated with intact endothelial cells. Following anion exchange chromatography, EP24.15 and EP24.16 activities were distinguished by sensitivity to the dipeptide inhibitor Pro-Ile (which was EP24.16-specific), cleavage of acetyl-neurotensin 8-13 (which was EP24.16-specific), and activation by dithiothreitol (which was EP24.15 specific). Using these findings, we determined that both EP24.15 and EP24.16 exist in soluble form, while the membrane-associated activity was primarily EP24.16-like. Thus, both EP24.15 and EP24.16 are expressed by aortic endothelial cells in culture, and may participate in bradykinin metabolism in the circulation.

Substrate Analogues Incorporating Beta-Amino Acids: Potential Application for Peptidase Inhibition.
Rebecca A Lew, Karen M Stewart, Patrick Perlmutter, C Hamilton, Shane Reeve, M Ursula Norman, Marie-Isabel Aguilar & A Ian Smith.

We hypothesized that substitution of alpha-amino acids at or around the scissile bond of a peptide substrate with beta-amino acids (containing an extra carbon in the peptide backbone) would confer resistance to proteolytic cleavage without necessarily abolishing enzyme binding. Indeed, such a stabilised analogue may act as a specific inhibitor of the peptidase. To test this possibility, we have synthesised a series of beta-amino acid-containing bradykinin (BK) analogues and examined their degradation by the soluble metalloendopeptidase EC 3.4.24.15. Inclusion of a beta-amino acid at or near the cleavage site of BK completely prevented degradation of the modified BK. Furthermore, such analogues could still act as competitive inhibitors. The affinities of the most potent peptides for recombinant EC 3.4.24.15 were only 1.5- to 2.5-fold lower than those of native BK. Interestingly, these analogues also acted as agonists at the B2 BK receptor in coronary artery segments, although their potencies were 2 to 3 orders of magnitude less than the native peptide.

In conclusion, substitution of beta-amino acids at the scissile bond can stabilise peptides against hydrolysis with only a small decrease in enzyme affinity. Thus, peptidomimetics incorporating beta-amino acids may be useful in the design and development of novel, specific, substrate-based peptidase inhibitors.

Subcellular Trafficking and Phosphorylation of Endothelin (ET)-Converting Enzyme-1.
Nathalie Tochon-Danguy, Walter G Thomas & A Ian Smith.

ET-1 is produced by the cleavage of its inactive precursor, big endothelin-1 by endothelin-converting enzyme (ECE). ECE is a type II integral membrane protein and a member of the metalloprotease family containing the classical HEXXH zinc binding motif. Three human ECEs have been identified to date; ECE-1, ECE-2 and ECE-3. The most abundant form, ECE-1, is expressed in at least four isoforms (ECE-1a, 1b, 1c and 1d), all encoded by the same gene but differently spliced. The four isoforms display similar kinetics but have different subcellular localities and tissue distribution. Several putative phosphorylation sites can be found in the cytoplasmic tail of ECE; some of which, when phosphorylated, may play a role in the regulation of ECE activity and/or trafficking.

We have shown that endogenous ECE-1 expressed in an endothelial cell line (EAhy926) as well as ECE-1c expressed in Chinese Hamster Ovary cells displayed a basal level of phosphorylation which could be increased following stimulation by phorbol esters. The level of ECE expression at the cell surface also appears to be modified following phorbol ester stimulation The target site for phosphorylation within the cytoplasmic tail has now been determined by site directed mutagenesis.. We are currently examining whether possible ligands and/or their receptors may be physiological mediators of the activation of protein kinase C and thereby regulate ECE-1 distribution in endothelial cells.

Grants and other funding

Ramacciotti special initiative grant for proteomics
$1,000,000

Wellcome Foundation large equipment grant.
N Hoogenraad, J Gorman, P Colman, AI Smith, M Berndt, $167,000

R&D START grant Endothelin Converting Enzyme Inhibitors for the Treatment of Cerebral Ischaemia
A Ian Smith, (with AMRAD corporation), 1998-2001, $2,000,000

NHMRC large equipment grant

Presentations

26th Lorne Conference on Protein Structure and Function, Lorne, Australia

4th Australian Peptide Conference, Lindeman Island, Australia

Annual meeting of Biological Psychiatry, Melbourne, Australia

4th International/17th American Peptide Symposium Peptide meeting, 2001 San Diego, California, USA

Korean Peptide Society Meeting, Seoul, Korea

Bio 2001, San Diego, USA

British Electrophoresis Society: From Biology to Pathology – the Protoemics Perspective, York, UK

Combio2001, Canberra, Australia

Students

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Research projects

Role of beta-arrestins in AT1 receptor function.
Walter Thomas, Hongwei Qian and Louisa Pipolo.

The aim of this study was to examine the physical association of arrestins – proteins involved in receptor internalisation and desensitisation – with the angiotensin receptor, AT1.

Methods included co-expression of wild type and mutated AT1 receptors and epitope-tagged arrestins in cells, immunoprecipitation of receptor and examination of the co-association of arrestins by Western blotting for the epitope-tag on arrestin.

We showed an association between AT1A receptors and arrestins which was agonist-driven and dependent upon a specific, phosphorylated motif within the receptor carboxyl-terminus.

States of angiotensin receptor AT1.
Walter Thomas and Hongwei Qian.

By comparing the signalling, phosphorylation and internalisation of wild type and mutated AT1 receptors in response to angiotensin and substituted analogues, we have provided evidence for multiple states of functional AT1 receptors.

To gain additional evidence for separate AT1 receptor states we applied a number of techniques: examination of AT1 receptor internalisation using a green fluorescent-tagged AT1 receptor and confocal microscopy, AT1 receptor phosphorylation using 32P-labelling and immunoprecipitation, and AT1 receptor signalling by Western blotting for activated MAP kinases. The work was done in Chinese hamster ovary cells stimulated with angiotensin or members of a series of substituted analogs.

The studied showed that separate, yet overlapping, contacts between the AngII peptide and the AT1A receptor selected/induced distinct receptor conformations that preferentially affect particular receptor outcomes. The requirements for AT1A receptor internalisation appear to be less stringent than receptor activation and signaling, suggesting an inherent bias towards receptor deactivation.

Epidermal Growth Factor Receptor (EGFR) transactivation in cardiomyocytes.
Walter Thomas, Hongwei Qian, Louisa Pipolo, Ross Hannan & Yves Brandenberger.

Angiotensin II (AngII) may cause cardiac hypertrophy via type 1 AngII receptors (AT1) on cardiomyocytes and through growth factors released from cardiac fibroblasts. Cardiomyocyte-specific AT1 receptor expression produces cardiac hypertrophy and remodeling in vivo, however is is difficult to identify the signals that mediate growth to AngII because the current in vitro model (cultured neonatal cardiomyocytes) expresses low levels of AT1 receptor.

We studied the effect of AngII in a modified in vitro model by expressing AT1A receptors in cultured neonatal cardiomyocytes using adenovirus.

AngII stimulated hypertrophy that was accompanied by the induction of the immediate-early response genes, c-fos and c-jun, and re-expression of atrial natriuretic peptide (ANP). AngII-induced activation of an ANP promoter-reporter was inhibited by the dominant/negative mutants, G-alpha-qI and N17Ras, indicating that hypertrophic signaling by the AT1A receptor is via heterotrimeric G protein coupling and downstream Ras pathways. AT1A-mediated cardiomyocyte hypertrophy and mitogen-activated protein kinase (MAPK) activation were inhibited by the MAPK kinase inhibitor, PD98059, and the EGFR kinase antagonist, AG1478, but not by PKC inhibitor, bisindolylmaleimide-1. Moreover, AngII-induced MAPK activation was prevented by treatment with a matrix metalloproteinase inhibitor, consistent with the tyrosine phosphorylation of the EGF receptor in response to AT1A receptor activation.

AngII directly promoted cardiac myocyte growth via AT1A receptors and EGF receptor transactivated MAPK signaling was important to this process.

Grants and other funding

Activation and regulation of angiotensin receptors
Walter G Thomas, National Heart Foundation of Australia, 2001-2002, $41,000

Presentations

26th Annual Conference on Protein Structure and Function, Lorne, Australia

Angiotensin Gordon conference Ventura, USA (invited speaker)

4th Australian Peptide Conference, Lindeman Island, Australia(invited speaker)

24th Annal Scientific Meeting of the Japanese Society of Hypertension, Osaka, Japan (invited speaker)

Renin Angiotensin Aldosterone Symposium, Melbourne, Victoria (invited speaker)

11th International Conference on Second Messenger and Phosphoproteins, Melbourne, Australia

Renin Angiotensin Aldosterone Symposium, Melbourne, Australia (invited speaker)

High Blood Pressure Council Meeting, Melbourne, Australia

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Research projects

A Randomised, Double-Blind Placebo Controlled Trial of Preoperative Coenzyme Q10 Therapy: Improved Outcomes in Coronary Artery Bypass Surgery.
S Pepe, W Lyon, S Marasco, M Wowk, F Sheeran, R Ou, JA Smith, A Pick, M Rabinov, B Davis, D Esmore & FL Rosenfeldt.

Coenzyme Q10 (CoQ10) is a component of cellular membranes, essential for antioxidant function and mitochondrial energy production which may have cardioprotective benefits. With the current study, we aimed to test whether pre-operative CoQ10 therapy: 1) increased CoQ10 content in atrial trabeculae and mitochondria; 2) improved mitochondrial respiration; 3) protected the myocardium in vitro against post-hypoxic contractile dysfunction; 4) attenuated intra-operative myocardial injury; 5) altered the length of hospital stay and/or 6) altered long-term quality of life measures.

Patients (n=122) were randomised, double blind, to receive CoQ10 (300mg/day) or placebo orally for at least seven days before elective surgery. On average, patients received 3 grafts during 60 min of aortic cross-clamp. Trabeculae were excised and mitochondria were isolated from discarded right atrial appendages. Follow up was assessed via SF-36 questionaire 12 months after surgery.

Therapy increased CoQ10 content of atrial trabeculae and isolated mitochondria, and improved the efficiency of mitochondrial respiration. The recovery of developed force after 30 min of hypoxia was greater in CoQ10-treated trabeculae. Patients receiving CoQ10 tended to have a shorter intensive care stay (1.4±0.1 vs 1.7±0.1 days, p=0.056) and total length of hospital stay (8.7±2.1 vs 6.8±0.7 days, p=0.044). The quality of life study indicated that physical well-being scores were significantly better for CoQ10-treated patients even 12 months after cardiac surgery, indicating that preoperative oral CoQ10 therapy was of benefit in relation to all parameters tested.

Enkephalin metabolism and coronary release during cardiac ischemia-reperfusion and ischemic preconditioning.
S Pepe, A Younès, J Caffrey & EG Lakatta.

Our studies have indicated that enkephalins are synthesised from proenkephalin, processed by and released from isolated, perfused rat hearts. The rate of synthesis exceeds the rate of release. This study was designed to evaluate the effects of ischemia and reperfusion on the cardiac turnover of endogenous proenkephalin and its main products, particularly during the cardioprotection produced by the natural phenomenon of ischemic preconditioning.

Isolated isovolumic hearts were perfused or submitted to 30-min total ischemia. Enkephalin peptides from tissue extracts and coronary effluent were separated and quantified. Proenkephalin, peptide B and Met enkephalin (ME)-arg-phe (MEAP) comprised 95% of extracted enkephalins. Ischemia and reperfusion lead to augmented coronary release of ME and MEAP. Post-ischemia, cardiac proenkephalin and ME concentrations decreased by 25% and 75% respectively while that of MEAP resembled the control value. The synthesis of new ME and MEAP decreased by 40% and 80%, respectively. When cardioprotection was invoked by ischemic preconditioning, proenkephalin degradation was augmented while cardiac levels of ME and MEAP were maintained.

These data suggest that post-ischemic reperfusion causes a loss of cardioprotective enkephalins, however cardiac enkephalin levels are preserved by ischemic preconditioning and are associated with reduced post-ischemic cellular damage and improved contractile performance.

Improved post-hypoxic contractile recovery and reduced oxygen demand after human myocardial delta-opioid peptide receptor activation.
S Pepe, F Sheeran, D Esmore & F Rosenfeldt.

Opioid peptides have been shown to be protective in animal models of cardiac reperfusion injury.
We tested whether stimulation of the delta-opioid peptide receptor (OPR) would increase post-hypoxic recovery of developed force (DF) in trabeculae isolated from right atria of humans undergoing coronary bypass surgery. Trabeculae were made hypoxic for 30 min and paced at 3Hz. Post-hypoxic recovery of DF (1Hz) was expressed as % of pre-stress values, untreated control (C). Some trabeculae received the specific delta-OPR agonists alone [D-Ala2-Leu5-enkephalin (DADLE), Met-enkephalin-Arg-Phe (MEAP)] or combined with naltrindol (NAL; a delta-OPR antagonist) or 5-hydroxy-decanoate (HD, a mitochondrial KATP channel blocker).

Recovery of DF after 30min reoxygenation was, as % pre-hypoxic values, C=39.3±5.1%; DADLE=69.5±4.6%*; MEAP=64.8±5.6%*; DADLE+NAL =44±3.1%; DADLE+HD=33.5±3.9% and oxygen consumption in unpaced, post-hypoxic trabeculae was, in ng O/min/mg protein, C= 648±74; DADLE=410±50*; MEAP=425±53*; DADLE+NAL=634±72; DADLE+HD=613±69 where * indicates p<0.05 vs C. The decrease in O2 consumption after delta-OPR agonists was restored by HD suggesting an involvement of mitochondrial KATP channels in this effect. In conclusion, delta-OPR stimulation protects against post-hypoxic contractile dysfunction and reduces O2 demand, suggesting that it increases energy efficiency.

Na+/H+ exchange inhibitor HOE-642 protects against post-ischemic human myocardial contractile dysfunction.
M Zhang, R Ou, S Pepe, W Lyon, M Wowk & F Rosenfeldt.

Steps to control calcium overload have dramatically improved protection of the heart during cardiac surgery. Inhibitors of Na+/H+ exchange prevent post-ischemic Ca2+ overload and associated contractile dysfunction in animal models. This study tested whether the Na+/H+ exchange inhibitor, HOE-642 was protective in an in vitro model of ischemia-reperfusion injury of human trabeculae.

Trabeculae were dissected from right atrial appendages discarded during surgery. Trabeculae were connected to tissue bath force transducers and electrically paced at 1 Hz in Ringer's solution. Tissues were subjected to 60min simulated ischemia in humidified N2. After 30 min of reperfusion, post-ischemic recovery of developed force was significantly improved by 1 micromolar HOE-642.

Expedition trial of cariporide.
F Rosenfeldt & D Esmore.

This project involved contributing to a double-blind, placebo-controlled multinational trial to investigate the effect of intravenous treatment with the Na+/H+ exchange inhibitor cariporide (HOE-642) on all-cause mortality and non-fatal myocardial infarction in patients at risk of myocardial necrosis during and after coronary artery bypass graft surgery. We enrolled our first patients late in 2001. The trial is due for completion in late 2003.

Grants and other funding

The senescent human myocardium: response to cardiac surgery and the protective benefit of coenzyme Q10
F Rosenfeldt, P Nagley and S Pepe, National Heart Foundation of Australia, 2000-2001, $44,100

The senescent human myocardium: response to cardiac surgery and the protective benefit of coenzyme Q10
F Rosenfeldt, P Nagley and S Pepe, Getz Bros & Blackmores Australia, 2001, $45,000
Endogenous cardiac enkephalin synthesis and coronary release
S Pepe, A Younes, J Caffrey and E Lakatta, National Institutes of Health Intramural Program (USA), 1999-2001, $60,000

Commercially funded research activities/clinical trials

Cariporide and human myocardial contractile dysfunction
F Rosenfeldt, S Pepe and R Ou, Aventis Pharmaceuticals, 2001, $30,250

Expedition trial of cariporide
F Rosenfeldt and D Esmore, Aventis Pharmaceuticals, 2001-2003, $14,000

Leukocyte filtration technology during and after cardiac surgery
R Salamonsen, F Rosenfeldt, and D Esmore, Pall Medical, 2001, $18,000

Presentations

Satellite Meeting to XVII World Congress of the International Society for Heart Research, Banff, Canada

XVII World Congress of the International Society for Heart Research, Winnipeg, Canada

74th Scientific Sessions of the American Heart Association, Los Angeles, USA

9th Congress of the International Association of Biomedical Gerontology, Vancouver, Canada

15th Bienniel Asian Congress on Thoracic and Cardiovascular Surgery, Mumbai, India

49th Annual Scientific Meeting of the Cardiac Society of Australia & New Zealand, Auckland, New Zealand

Joint Meeting of the Satellite to XXXIV World Congress of the International Union of Physiological Sciences and the Annual Scientific Sessions of the International Society for Heart Research, Brisbane, Australia
International Conference of the Society for Free Radical Research, Sydney, Australia

Visiting scientists

Prof. Chris Munsch, Leeds Royal Infirmary, Leeds, UK

Students

PhD
Francis J Miller MBBS (Monash)
Olivier van den Brink MD (Amsterdam)

MD
William Lyon MBBS (Flinders)

Master of Surgery
Silvana Marasco MBBS (Monash)

Experimental Cardiology

HEADS
Anthony M Dart
BA, DPhil, BMBCh (Oxford)
MRCP, FRCP

Xiao-Jun Du
MBBS (Chongqing),
MMed (Xian) PhD (Edinburgh)

SENIOR SCIENTIFIC
Elodie Percy BScHons (Melbourne)

Research projects

Age-dependent development of cardiomyopathy phenotype in a strain of mouse with cardiac-targeted overexpression of beta2-adrenergic receptors.
AM Dart, XM Gao & XJ Du

We have recently demonstrated that male and female transgenic mice that over-express the beta2 adrenergic receptor differ significantly in the severity of cardiomyopathy. Females have considerably less functional and pathological abnormalities compared with males, which leads to to better survival in the females.

Cardiac phenotype in the S100A1 knock-out mouse.
J Heierhorst*, B Kemp* & XJ Du.
(* St Vincent's Institute of Medical Research)

We have observed a stress-dependent cardiac phenotype in S100A1 knock-out mice. This study is the first to demonstrate the importance of S100A1 protein in the maintenance of cardiac function under acutely- and chronically- stressed conditions,

implying that a down-regulation of S100A1 in the hypertrophied and failing heart may contribute to the decreased contractility of such diseased hearts.

Cardiac function in the relaxin knock-out mouse.
G Tregear* & XJ Du. (Howard Florey Institute)

We have observed that relaxin is expressed in the heart, and that male relaxin knockout mice develop cardiac diastolic dysfunction due to increased myocardial interstitial collagen content. Further studies are in progress to examine the role of relaxin in cardiac diseases in relation to its activities on cardiomyocytes and on the extracellular matrix.

Cardiac rupture following myocardial infarction.
AM Dart, XM Gao & Du XJ.

Cardiac rupture accounts for approximately 12% of in-hospital deaths in patients with acute myocardial infarction (AMI). There has been no animal model of rupture for research use. Our recent findings show that rupture occurs in mice with AMI, which mimics human cardiac rupture in many aspects. We have also demonstrated a significant reduction in the mechanical strength of the infarcted myocardium.

Grants and other funding

HIV infection, its treatment and vascular disease
A Dart, C Gatzka and A Mijch, Alfred Research Trust, 2001, $25,000

Role of endothelin in the disease progression of patients with hypercholesterolaemia
J Chin-Dusting and A Dart, Alfred Research Trust, 2001, $23,728

Effects of hormone replacement therapy on arterial stiffness in women with coronary disease
B Kingwell, A Dart and P Komesaroff, Australian Menopause Society, 2001, $20,000

A Coach for cardiovascular patients in every hospital: coaching patients on achieving cardiovascular health
M Jelinek, J Best, A Dart and D Hare, Victorian Health Promotion Foundation, 2000-2001, $75,000

Change in arterial properties and its effect on pressure transfer with blood pressure and heart rate
C Gatzka, J Cameron and A Dart, National Heart Foundation of Australia, 2001, $33,919

The role of fibrillin-1 in the structural basis of large artery stiffening
B Kingwell, M West and A Dart, National Heart Foundation of Australia, 2000-2001, $85,527

Travel Grant
XM Gao and E Percy, National Heart Foundation, 2001, $1,500

Commercially funded research activities/clinical trials

Efficacy and safety of the oral direct thrombin inhibitor H 376/95 compared with dose-adjusted warfarin
A Dart, Astra Zeneca, 2001, $11,916

An open label, randomised phase 111b, parallel group switching study to compare the efficacy & safety of lipid lowering agents atorvastatin, pravastatin, simvastatin & rouvastatin
A Dart, Astra Zeneca, 2001, $20,702

Evaluation of the antianginal efficacy & safety of oral chronic administration of ivabradine compared to atenolol in patients with stable effort angina pectoris
A Dart, Servier, 2001, $4,000

A 4 year, double blind, placebo controlled study of atorvastatin as secondary prevention of CHD in patients with type 11 diabetes
A Dart, Pfizer, 2001, $50,106

A multinational, multicentre, randomised, double blind, placebo controlled clinical trial
A Dart, Bayer Pty Ltd, 2001, $14,315

Pravastatin or Atorvastatin evaluation and infection therapy
A Dart, Bristol-Myers Squibb Co, 2001, $26,295

The effects of LDL-cholesterol lowering beyond currently recommended minimum targets on CHD recurrence in patients with pre-existing CHD
A Dart, Pfizer, 2001, $68,174

S16257 and Cardiomyopathic Phenotype in beta2-AR Transgenic Mice
A Dart and XJ Du, Servier, 2001, $157,161

Visiting scientists

Dr Mark Tuner, Cardiac Academic Unit, St Mary Hospital, Empire College, London, UK

Presentations

3rd Cardiovascular-Pharmacology Symposium, Lubeck, Germany

4th International Workshop, Paris, France

Lipids and Vascular Disease, Diabetic Complications, Satellite Symposium to the IUPS Meeting

Surgery Vs Angioplasty. Future Trends, CVP SIG of Australian and New Zealand College of Anaesthetists.

The 17th World Congress of International Society for Heart Research, Winnipeg, Canada

Large Vessel Arterial Disease. New Approaches to Cardiac Therapeutics-Pharmacology , Physiological and Clinical, Brisbane, Australia

Australia/New Zealand International Society for Heart Research Annual Conference

Students

PhD
James Shaw MBBS (Melbourne) FRACP
Mark Krawczysyn MBBS (Melbourne) PRACP
Xiaoming Goa MBBS (Xinjiang

Undergraduate
Tzeping Tan Advanced Medical Science (Melbourne)

Human Neurotransmitter

HEAD
Murray Esler
BMedSci, MBBS(Melbourne), PhD (ANU) FRACP

SENIOR SCIENTIFIC
Jacqueline Hastings PhD (Deakin)
Gavin Lambert PhD (Monash)
Elisabeth Lambert PhD (Paris)
John Power BVSc (Queensland), PhD (Monash)
Marcus Schlaich MD (Freiburg)
SENIOR SCIENTIFIC
Alison Brown BSc (Monash)
Florentia Socratous BSc (LaTrobe)

Research projects

Hypertension – The neurobiology of essential hypertension.
Marcus Schlaich, Elisabeth Lambert, Magdalena Rumantir, David Kaye, Gavin Lambert Sue Luff (Monash) & Murray Esler.

In a substantial proportion of patients, essential hypertension is neurogenic, with high rates of spillover of noradrenaline from the heart and kidneys, attributable in part, at least, to increased sympathetic nerve firing rates. Other possible causes may be an increase in the density of sympathetic innervation, facilitation of neuronal noradrenaline release by adrenaline released from sympathetic nerves as a cotransmitter, and impairment of neuronal noradrenaline reuptake.

We are performing morphometric comparisons of sympathetic nerve density in dorsal hand vein biopsies from essential hypertensive patients and healthy volunteers and studying nerve growth factor gene expression. In a separate group of hypertensive patients we are examining multi- and single-unit firing in the sympathetic outflow to the skeletal muscle vasculature and cardiac adrenaline cotransmission and noradrenaline transporter function.

Hypertension – L-arginine transport and its role in impaired endothelium dependent vasodilation in essential hypertension.
Marcus Schlaich & David Kaye.

Evidence suggests endothelial dysfunction is a cause of essential hypertension rather than a consequence of the condition and that it may involve a defect in the L-arginine/NO pathway, since arginine supplementation can restore endothelial function. Endothelium dependent vasodilation is also influenced by the sympathetic nervous system, which is particularly active in young people with high blood pressure. It is not known whether increased sympathetic activity is related to impaired endothelial function present in young hypertensive and normotensive subjects who have a genetic predisposition for hypertension.

We have commenced a study to examine muscle sympathetic nerve activity, endothelium - dependent and -independent vasodilation and regional L-arginine clearance in people with high blood pressure and normal blood pressure.

Heart Failure – Reflex control of sympathetic activity.
David Kaye, Anne Aggarwal, Gavin Lambert, Jacqueline Hastings & Murray Esler.

The mechanism of the sympathetic nervous activation present in heart failure is unknown, although we and others have obtained evidence that elevated intracardiac pressures are involved. In heart failure patients, unloading the heart with drugs or by the application of negative pressure to the lower body reduces cardiac sympathetic tone, whereas in healthy people, these interventions cause sympathetic stimulation. We have also found that increasing the intrathoracic pressure with continuous positive airway pressure (CPAP) – widely used for obstructive sleep apnoea – lowers cardiac sympathetic tone in heart failure patients.

Heart Failure – Myocardial sympathetic nerve density and its regulation.
David Kaye, John Power, Melissa Byrne & Murray Esler.

Histological studies have indicated reduced myocardium sympathetic neuronal density in heart failure, although neurochemical measures indicate increased levels of cardiac sympathetic firing and neurotransmitter release. We have shown that the lower density of neurons in human and experimental heart failure may arise from feedback inhibition by the sympathetic transmitter, noradrenaline, of cardiac expression of nerve growth factor (NGF). The under-expression of NGF by cardiomyocytes in the failing heart may represent an adaptive response to exposure of the myocardium to high, potentially toxic levels of noradrenaline.

Heart Failure – Influence of atrial fibrillation.
John Power & Melissa Byrne.

Atrial fibrillation (AF) commonly accompanies heart failure and is associated with a poorer prognosis. Recently it has been shown that chronic lone atrial fibrillation is associated with an increased level of apoptosis in ventricular myocytes. In a collaborative study with Professor Allessie from The Netherlands, John Power and his group are currently examining AF, electrically triggered, in the sheep heart failure model, analysing the effects of AF on cardiac sympathetic activity, myocardial performance, and myocardial histology and neurochemistry utilising sequential endocardial biopsies.

Heart Failure – Cardiac cachexia.
John Power & David Kaye.

Disability in human heart failure is in part due to a decrease in cardiopulmonary reserve which limits exercise capacity. Skeletal muscle dysfunction also contributes to heart failure symptoms. Weight loss in heart failure patients can be extreme, justifying use of the term "cardiac cachexia". But unlike the cachexia in other clinical contexts, in heart failure, the loss of tissue mass is in skeletal muscle not adipose tissue.

We have recently commenced an investigation into the mechanisms of cardiac cachexia in the sheep paced heart failure model, in which weight loss is a feature. DEXA methodology will be used to study body composition during the development of heart failure. We will measure total energy and protein balance and gene expression and activity of the mitochondrial uncoupling protein, UCP 3, in skeletal muscle biopsy specimens.

Heart Failure – Influence of experimental left bundle branch block.
John Power & Melissa Byrne.

An adverse effect of ECG left bundle branch block (LBBB) is heart failure in humans, thought to be due to lack of synchrony in the ejection from the ventricles. It is not known whether LBBB initiates the development of heart failure or is secondary to cardiac structural change as a consequence of the failure. In a two-year study, using intracardiac ablation, we are examining LBBB in normal sheep by sequential assessment of any effects on cardiac function, and, if there are, whether these are mediated by cardiac sympathetic overactivity.

The neurobiology of psychosomatic heart disease – Panic disorder.
Marlies Alvarenga, Elisabeth Lambert, Gavin Lambert, David Kaye, David Barton & Murray Esler.

Until recently, panic disorder was considered distressing and disabling but not a risk to life. However, it is now known that patients with panic disorder have a 3- to 6- fold increased risk of myocardial infarction and sudden death. This may be due to ventricular tachyarrhythmias induced by cardiac sympathetic activation during a panic attack, or activation of platelets by high plasma catecholamine concentrations leading to thrombogenesis.

To determine which pharmacological measures for prophylaxis of sudden death should be considered, we are continuing to delineate the CNS neurochemical mechanisms underlying panic disorder, to quantify the cardiac sympathetic outflow during panic attacks, to examine the cardiac and sympathetic baroreflex and to establish whether platelet activation is present. We are continuing to compare the effects of pharmacological therapy with selective serotonin reuptake inhibitors and cognitive behavioural therapy.

The neurobiology of psychosomatic heart disease – Depression and the heart: identifying the relation between affective disorders and coronary heart disease.
Gavin Lambert, David Barton, Elisabeth Lambert, Markus Schlaich, Elizabeth Gardner, Anne Aggarwal, David Kaye & Murray Esler.

Major depression is ranked fourth among the 10 leading causes of the global burden of disease and, if epidemiological projections are correct, by 2020 it will reach second place. Recent well-conducted prospective studies demonstrate unequivocally that patients with endogenous depression are at increased risk of developing coronary heart disease.

In this project we aim to:

(1) Resolve whether the clinical manifestation of depression is associated with abnormalities in brain neurotransmitter turnover.
(2) Determine why patients with depression are at elevated risk for the development of coronary heart disease.We will examine brain neurotransmitter turnover, sympathetic nervous activity, platelet reactivity, and cardiac (vagal) and sympathetic baroreflex sensitivity before and after antidepressant drug therapy or cognitive behavioural therapy.

(3) Measure platelet reactivity, whole body noradrenaline kinetics, cardiac specific enzymes, cardiac repolarisation disturbances and spontaneous cardiac (vagal) baroreceptor sensitivity in patients with severe depression before and after electroconvulsive therapy.

(4) Investigate the association between myocardial infarction, depression, quality of life and mortality.

Obesity and obesity-related hypertension.
Glen Weisner, Jacqueline Hastings, Nina Eikelis, Magdalena Rumantir, David Kaye, Elisabeth Lambert & Murray Esler.

Our wide-ranging research projects in obesity have involved studies of leptin and its influence on the sympathetic nervous system. We discovered that leptin was produced in the myocardium of both the healthy and failing human heart and released from the failing heart into the coronary sinus. In cultured rat myocytes our preliminary observation is that concentrations of leptin in the physiological range promote the uptake and metabolism of palmitate but are without effect on glucose uptake. Fatty acids such as palmitate are the principal oxidative substrate of the myocardium.

Given the worldwide epidemic of obesity, and the pivotal importance of obesity as a cause of hypertension, it is surprising that knowledge of the mechanisms by which blood pressure is elevated by obesity is so rudimentary. Our own observations suggest that hypertension in the obese is primarily neurogenic. Renal sympathetic nervous activity is markedly increased in obesity. We are investigating the role of leptin in the activation of renal sympathetic outflow and whether the increase in CNS serotonin turnover in obesity contributes to the high sympathetic tone. We are also studying the predisposing factors, genetic or otherwise, for why obesity leads to hypertension in about 50% of cases.

Grants and other funding

Cardiac nerve growth factor expression: pathophysiologic alterations in congestive heart failure
D Kaye and M Esler, 2001-2002, $36,880, National Heart Foundation of Australia

Point of origin postdoctoral funding for clinical scientists
M Schlaich, 2001-2002, $40,000, German Medical Research Foundation (Deutsche Forschungs Gemeinschaft)

Sympathetic nervous system activity and aging
M Esler, D Seals and G Jennings 1997-2001, $378,431, National Institute of Health, USA

Commercially funded research activities/clinical trials

Investigator initiated drug trial
M Esler, 2000-2002, $206,000, Solvay Pharmaceutical Company

The projects conducted by Dr John Power were funded by a number of companies: Guidant Corporation, Boston Scientific, Cardiac Dimensions Inc, Converge Medical Inc, Acorn Cardiovascular Inc (Study Grant)

Visiting Scientists

Professor Irv Zucker, University of Nebraska, USA

Dr David Kass, Division of Cardiology, Department of Medicine, Professor of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA.

Dr Xavier Gonzales, Vice President Spiration Inc, Redmond WA, USA.

Dr David Reuter, Cardiac Dimensions Inc, Kirkland WA, USA.

Dr Huy Than, Boston Scientific, San Jose, CA, USA

Professor Mikael Elam, Department of Neurophysiology, Gothenburg University, Gothenburg, Sweden

Presentations

International Symposium on Angiotensin Receptor Blockers, Monte Carlo (Invited speaker)

International Meeting on Presynaptic Receptors, Madeira (Invited speaker)

Symposium on Adrenergic Mechanisms in Heart Failure, Lubeck, Germany (Invited speaker)

European Society of Hypertension Meeting, Milan, Italy (Invited speaker)

Franco-Australian Meeting on Hypertension, Corsica, France (Invited speaker)

2nd Internation Society of Obesity Meeting, Berlin, Germany (Invited speaker)

European Cardiology Conference, Stockholm, Sweden

2nd Asian Pacific Congress on Hypertension, Thailand (Invited speaker)

Annual meeting of the North American Association for the Study of Obesity (NAASO), Quebec City, Canada (Invited speaker)

American Society of Hypertension Meeting, San Francisco, USA (Invited speaker)

American Heart Association, Anaheim, USA

American College of Cardiology, Orlando, USA

International Catecholamine Symposium held in Kyoto, Japan (Invited speaker)

International Union of Physiological Sciences, Christchurch, New Zealand (Invited speaker)

Satellite meeting of the 2001 International Congress of Physiological Sciences – Cardiorenal Control in Health and Disease, Queenstown, New Zealand (Invited speaker)

Satellite meeting of the 2001 International Congress of Physiological Sciences – Central mechanisms of cardiovascular control: cellular, molecular and integrative aspects, Sydney, Australia

New Perspectives in Cardiovascular Disease and its Treatment, Sydney, Australia (Invited speaker)

Annual meeting High Blood Pressure Research Council of Australia

Australian Society for Cellular and Molecular Gerontology, Melbourne, Australia

Students

PhD
Marlies Alvarenga BScHons (Monash)
David Barton MBBS (NSW)
Melissa Byrne BScHons (RMIT)
Nina Eikelis BScHons (Monash)
Magdalena Rumantir DM, BMedSc (Jakarta)
Glen Weisner BScHons (Melbourne)

Molecular Hypertension

HEAD
Zygmunt Krozowski
BScHons (WA), PhD (Sydney)

SENIOR SCIENTIFIC
Zhonglin Chai PhD (Monash)
PROFESSIONAL & TECHNICAL
Varuni Obeyesekere BScHons (Monash)
Michelle Cinel CertVetNurs, AssocDipAppSc (AnimalTech)
Carla Duarte BScHons (LaTrobe)

Research projects

Characterization of a novel 17beta-dehydrogenase, 17betaHSDXI.
Phillip Brereton, Zhonglin Chai, Varuni Obeyesekere, Takashi Suzuki, Hironobu Sasano, David Kaye, Richard Safery, Peter Fuller, Carla Duarte, Genevieve Escher & Zygmunt Krozowski.

We identified a novel isoform of 17beta-hydroxysteroid dehydrogenase, 17betaHSDXI, that has conserved domains of the short chain alcohol dehydrogenase superfamily. This project aimed to discover the biological roles of this enzyme. Activity of the cloned extract was tested on a range of substrates and antibodies were raised against a synthetic peptide and to detect the location of cellular 17betaHSDXI in human tissues.

This enzyme converts 3alpha-Adiol to androsterone and is inhibited by retinoids, but not carbenoxolone. Three-alpha-Adiol has been implicated in supporting gestation and modulating GABA receptor activity. Of proteins with known function, 17betaHSDXI had 30% identity with the retinol metabolising enzyme retSDR1. Northern blot analysis showed high levels of expression in pancreas, kidney, liver, lung, heart, adrenal and ovary.

Immunohistochemical staining for 17betaHSDXI was strong in steroidogenic cells, sebaceous gland, Leydig cells and granulosa cells of the dominant follicle and corpus luteum. In the adrenal, staining was strongest in the glomerulosa and increased after birth. Studies on regulation of 17betaHSDXI in mouse Y1 cells showed that cAMP downregulates enzymatic activity (40% versus 32%, $p<0.05$) and reduces gene expression to undetectable levels. The addition of all trans retinoic acid with cAMP further decreased activity (32% versus 23%, $p<0.05$). Real time PCR studies showed significantly less 17betaHSDXI message in heart failure samples (1.0 vs 0.83, $p=0.021$). These results suggest a role for 17betaHSDXI in androgen metabolism during steroidogenesis.

Preparation of recombinant adenoviruses expressing 17betaHSDXI and other related proteins.
Zhonglin Chai, Carla Enriquez & Zygmunt Krozowski.

This project aimed to prepare recombinant adenoviruses to transduce genes to express functional 17betaHSDXI and related proteins in vitro and in vivo.

The coding region of 17betaHSDXI cDNA was tagged with two consecutive c-Myc epitopes at the N-terminus. The coding DNA fragment of Myc-17betaHSDXI was subcloned into pAdTrack-CMV shuttle vector and recombined with the adenovirus backbone plasmid, pAdEasy-1, through recombination in E coli strain BJ5183. To produce infectious viruses, the recombinant plasmid was linearised and transfected into HEK293 cells. The technology is being applied to a number of other proteins, including 17betaHSD1 and 2.

We concluded that stable adenoviral expression of dehydrogenases is readily achievable and will facilitate in vivo and in vitro studies of novel enzymes found in the heart.

Expression of sterol 27-hydroxylase (CYP27A1) and cholesterol efflux.
Genevieve Escher, Zygmunt Krozowski & Dmitri Sviridov.

The likely rate-limiting step of reverse cholesterol transport which removes excess cholesterol from extrahepatic tissues is cholesterol efflux. Cholesterol efflux occurs by transfer of cholesterol to lipid -free or –poor apolipoprotein A-I (ApoA-1) or by the transfer of cholesterol from plasma membranes to lipidated apoA-I or mature high-density lipoprotein (HDL). The aim of this study was to determine the effect of CYP27A1, which adds a hydroxyl group to make cholesterol more polar, on the rate of cholesterol efflux from mammalian cells.

CHOP cells transfected with CYP27, or mock-transfected for controls, were labelled with radioactive cholesterol and the efflux of cholesterol to or human plasma was measured. Transfection with sterol 27-hydroxylase stimulated cholesterol efflux to both acceptors by 2-3-fold. It had no effect on efflux in the absence of apoA-1 or plasma.

Light and electron microscopy localization of the 11beta-hydroxysteroid dehydrogenase type I (11betaHSD1) enzyme in the rat.
Phillip Brereton, Rosemary van Driel, Fariha Suhaimi, Kaori Koyama, Rodney Dilley & Zygmunt Krozowski.

Using immunohistochemical methods, we aimed to identify the various cell types containing the glucocorticoid activator, 11betaHSD1.

The enzyme 11betaHSD1 converts cortisone to cortisol in humans, and 11-dehydrocorticosterone to corticosterone in rodents. We used an immunopurified polyclonal antibody, RAH113, to localize 11betaHSD1 in a wide range of rat tissues. Staining for 11betaHSD1 in the liver was most intense around the central vein and decreased radially. In the lung, the highest levesl of 11betaHSD1 were found in the interstitial fibroblast and those in the type II pneumocyte were an order of magnitude lower. 11beta HSD was found in the proximal tubules of the renal cortex, in the interstitial cells of the renal medulla and papilla, in the adrenal glomerulosa and medulla, in parietal cells of the fundic region of the stomach, in the interstitial fibroblasts of the endocardium and in the adventitial fibroblasts of blood vessels.

Electron microscopy of lung and kidney interstitial cells showed that 11betaHSD1 was found in the endoplasmic reticulum and the nuclear membrane. The presence of 11betaHSD1 in discrete cell populations suggest that it may facilitate intracrine and paracrine glucocorticoid action in addition to its role of maintaining circulating glucocorticoids via its activity in the liver.

Visiting scientists

Dr Simon Slight, Columbia, USA

Dr Genevieve Escher, Berne, Switzerland

Presentations

Genome Conference, Lorne, Australia

Aldosterone Conference, Denver, USA

Endocrine Society Meeting, Denver, USA

Jo Guiliano Molecular Neurocardiology

HEAD
David M Kaye
MBBS, PhD (Monash)
FRACP, FACC

PROFESSIONAL & TECHNICAL
Sara Gruskin BSc (Murdoch), PGDipSc (Western Australia)
Samara Cairns BScHons (Melbourne)
Belinda Smirk BSc(MedSc)Hons (LaTrobe)

Research projects

Sympathoadrenergic mechanisms in heart failure.
David Kaye & Murray Esler.

This project represents ongoing work supported by a 5-year Wellcome Trust Senior Research Fellowship to Dr Kaye.

The key aims of the project are to establish the cellular and molecular basis for alterations in sympathetic nerve activity and the attendant high levels of catecholamine release that we have shown to be associated with poor outcome in heart failure patients.
As part of this project, we have used a broad suite of cellular and molecular techniques and have also performed several studies in patients. Basic studies have continued to focus on the role of nerve growth factor in the failing heart, and on the function of the noradrenaline transporter in the failing heart. Our studies in humans have focused on the mechanisms that control catecholamine release. Our techniques have relied on the use of the noradrenaline spillover method combined with the use of regional blood sampling and the administration of a range of pharmacological and physiological stimuli.

Nerve growth factor (NGF) as therapy in heart failure.
David Kaye.

As an extension of the studies described above, we have commenced a more detailed study of the secretion of NGF by cardiomyocytes, and more particularly in exploring the possibility that NGF may play a therapeutic role in heart failure. These studies will examine the local and systemic delivery of NGF as well as examining the structure and function of the heart in transgenic mice that express high levels of NGF in the heart.

Cellular, molecular and functional characterization of L-arginine transport in heart failure and hypertension.
David Kaye, Marcus Schlaich & Jaye Chin-Dusting.

This project combines a series of studies into the role of alterations in L-arginine transport (the precursor of nitric oxide) by the endothelium and myocardium. In patients, we have studied the capacity of the failing heart to extract extracellular L-arginine and have characterized the expression of the L-arginine transporter in failing myocardial samples.

Other studies in humans have covered a range of intervention studies, including the acute modulation of L-arginine transport in vivo and the long term effects of interventions such as exercise training and lipid lowering. We have also concentrated on defects, possibly genetic, in this pathway in patients with high blood pressure.

At the basic level, our studies are currently aimed at determining the mechanism by which neurohormones and cytokines modulate L-arginine transport, and at assessing how cells regulate the location of the L-arginine transporter.

Grants and Other Funding

Wellcome Trust Senior Research Fellowship
David Kaye, Wellcome Trust 1998-2002, $150,000

Nerve growth factor (NGF) as therapy in heart failure
David Kaye, National Heart Foundation, 2001-2002, $43,000

Commercially funded studies also form a component of the laboratory's activities, at present these are subject to 'commercial in confidence'.

Conferences attended

David Kaye and Melinda Parnell: American Heart Association Meeting, Anaheim, USA

David Kaye Heart Failure Society of America Meeting, Washington, USA

Students

PhD
Melinda Parnell BScHons (Monash)
Belinda Ahlers BScHons (James Cook)
– with Dr Jaye Chin-Dusting
Dr A Aggarwal MBBS (Melbourne)
Belinda Smirk BScMedSciHons (LaTrobe)

Neurophysiology

HEAD
Geoffrey A Head
BScHons (Melbourne), PhD (Monash)

SENIOR SCIENTIFIC
Dmitry N Mayorov BScHons, PhD (Moscow)
PROFESSIONAL & TECHNICAL
Sandra L Burke BScHons (Sydney), MSc (Monash)
Shirley J Godwin BAppSc (RMIT) (to February 2001)
Sandy Rogers BSc (Lincoln), MSc (Christchurch)

Research projects

Males, females and seasonal variation in ambulatory blood pressure recordings: analysis by new double logistic method.
GA Head, CM Reid, EV Lukoshkova & GL Jennings.

This project was designed to determine the rate of change in systolic (SBP), diastolic blood pressure (DBP) and heart rate (HR) from the day to night transition separately from the night to day transition by applying a new logistic curve-fitting procedure to ambulatory blood pressure (ABP) recordings from male and female subjects at different times of the year. ABP was recorded over 24hr from 71 male and 77 female subjects aged 54 years (range 23 to 87). Recordings from the 6 hotter months (Oct-Mar) were compared to those from the colder months (Apr-Sep).

All perameters showed a similar pattern of higher day and lower night values. The SBP differences were 41 mmHg for females and 38 mmHg for males. DBP and HR day/night differences were also similar in males and females, however absolute values of DBP were 3 mmHg lower in females than males during the day and the night. The early morning increases in SBP, DBP and HR were similar in males and females during both the cooler and warmer months. In females, the HR rose in the morning 70% more quickly than it fell in the evening. The corresponding difference in males was 44%. SBP and DBP rose and fell at the same rate.

The SPRINT study and others have shown similar incidences of morning peak in myocardial infarcts in males and females, suggesting that the rate of morning rise in BP may be a better predictor of cardiac events than absolute levels of BP.

Analysis of 24 hour blood pressure recordings in normotensive and hypertensive rats.
GA Head, SJ Chea, DN Mayorov, AM Arabia, M Van den Buuse & EV Lukoshkova.

We have developed a new logistic equation which enables measurement of the diurnal rates of transition in cardiovascular variables between active and sleep periods separately. This study aimed to apply the logistic method to telemetry recordings of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and activity of normotensive Sprague Dawley (SD) rats and spontaneously hypertensive rats (SHR).

Hourly values were determined for 5 SD rats and 11 SHR over 3 days for 3 weeks and fitted to a 6 parameter double logistic equation.

SHR had higher SBP and DBP, lower HR than SD. All cardiovascular parameters showed a similar pattern of higher awake and lower sleep values with the differences being 15 ± 1 mmHg for SBP, 77 ± 2 b/min for HR and 44 ± 2 units for activity in SHR. Awake/asleep differences were less for SBP (9 ± 1 mmHg) but greater for HR (98 ± 9 b/min) in SD

rats. The rates of transition between awake and sleep periods were similar for all parameters in SD, but, except for DBP, showed a non-symmetrical diurnal pattern for SHR. HR rose almost twice as fast in the evening period as it fell in the morning. There was also a greater rate of evening increase in SBP in SHR compared to SD. Compared with normotensive SD rats, SHR showed a greater rate of increase in activity during the arousal period which may be associated with the asymmetric diurnal pattern observed for SBP and HR.

Effect of chronic perindopril on 24 hour blood pressure recordings in SHR.
GA Head, SJ Chea, DN Mayorov & EV Lukoshkova.

This study aimed to assess the effects of chronic administration of the angiotensin converting enzyme inhibitor, perindopril, on telemetry recordings of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and activity in SHR.

Ten adult SHR with telemetry implants were treated with perindopril at 1 mg/kg/day in the drinking water for 2 weeks and compared with 11 control animals. Values were recorded hourly for each animal over 3 days at the end of each week for 2 weeks before and during treatment. The data were fitted to a 6 parameter double logistic equation which estimates the rate of change of blood pressure between awake and asleep plateaus.

During treatment, perindopril reduced SBP and DBP by 21 mmHg and 16 mmHg respectively while HR was increased by 13 b/min. The effect of perindopril treatment was similar during the active period (night) and during the inactive period (day). Locomotor activity was not altered by perindopril. The rate of increase in SBP, DBP and HR from asleep to awake was similar before and during perindopril.

In conclusion, chronic perindopril treatment had a marked hypotensive action in adult SHR which was similar during active and inactive periods and did not alter the arousal-induced surge in blood pressure.

Chronic rilmenidine selectively reduces arousal blood pressure during 24 hour recordings in SHR.
GA Head, SJ Chea, DN Mayorov & EV Lukoshkova.

This study examined the effects of chronic administration of the antihypertensive agent, rilmenidine, on telemetry recordings of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and activity in SHR.

Ten adult SHR with telemetry implants were treated with rilmenidine 2 mg/kg/day in the drinking water for 2 weeks and compared with 11 control animals. Values were recorded hourly for each animal over 3 days at the end of each week for 2 weeks before and during treatment. The data were fitted to a 6 parameter double logistic equation which estimates the rate of change of blood pressure between awake and asleep plateaus.

Rilmenidine reduced SBP, DBP and HR by 7.4 mmHg, 6.1 mmHg and 14 b/min respectively. The main effect of rilmenidine was during the active period at night. Thus, rilmenidine significantly reduced the diurnal night to day difference. The rate of increase in SBP was 50% less and DBP 32% less with rilmenidine. The rates of decrease in cardiovascular parameters during the transition from awake to asleep values was unchanged.

The greater effect of rilmenidine in the active period may reflect the greater sympathetic vasomotor drive at this time.

AT1-receptors and glutamate receptors in the rostral ventrolateral medulla (RVLM) mediate the sympathoexcitatory response to acute stress in conscious rabbits.
DN Mayorov & GA Head.

We examined the effects of microinjections into the RVLM of non-selective angiotensin II (AII) receptor antagonist, sarile, or AT1-receptor antagonist, losartan, on the arterial pressure (AP) and renal sympathetic nerve activity (RSNA) to airjet stress in conscious rabbits.

Airjet stress caused increases in AP and RSNA, which stabilised after several minutes at +9 mmHg and +18 nu, respectively. Sarile decreased the AP and RSNA responses to airjet stress by 48% and 56%,

respectively. By contrast, losartan did not alter the onset of AP and RSNA responses to airjet, but abolished these responses over time. Microinjections of AII antagonists adjacent to the RVLM had no effect on responses to airjet stress. Pretreatment with the excitatory amino acid (EAA) receptor antagonist, kynurenic acid, markedly decreased the onset of AP and RSNA responses to airjet, but had little effect on the stable level reached by these responses.

These results suggest that the RVLM conveys excitatory environmental influences to the sympathetic nervous system in conscious animals. The EAA receptors in the RVLM are important in initiating the pressor and sympathoexcitatory responses to acute stress, while angiotensin AT1-receptors may play a critical role in maintaining these responses.

Glutamate receptors in the rostral ventrolateral medulla (RVLM) modulate sympathetic baroreflexes in conscious rabbits.
DN Mayorov & GA Head.

Studies in anaesthetised animals may have led to underestimation of the importance of excitatory amino acid (EAA) neurotransmission in the RVLM in mediating sympathetic baroreflexes. We re-examined this experimental situation, comparing conscious and anaesthetised rabbits.

Microinjection of the EAA receptor antagonist kynurenate into the RVLM did not affect resting RSNA, arterial pressure or heart rate (HR). Kynurenate decreased the gain of the RSNA baroreflex by 45%. Injection of kynurenate adjacent to the RVLM had variable effects on the measured parameters, depending on the site of injection. Pentobarbitone anaesthesia reduced the gain and range of the RSNA baroreflex by 70% and 50%, respectively and microinjection of kynurenate into the RVLM under these conditions caused no further reduction in gain.

Endogenously-released EAA neurotransmitters in the RVLM may be important in modulating sympathetic baroreflexes. Anaesthesia masks the functional significance of EAA in the RVLM in controlling the baroreflexes.

Evaluation of spontaneous sympathetic baroreflex sensitivity in conscious rabbits.
DN Mayorov, EV Lukoshkova & GA Head.

We evaluated the spontaneous sympathetic baroreflex gain in rabbits at rest and during sympatho-activation with hypoxia or CNS stimulation.

The RSNA baroreflex gain was estimated in conscious rabbits by using a novel application of the sequence method and comparing it to the phenylephrine and nitroprusside-induced ramp changes in arterial pressure (AP) before and during i) hypoxia and ii) microinfusion of glutamate into the pressor region of the RVLM.

In control conditions, the sequence gain was much higher than maximal ramp gain. The increase in RSNA (+60%) during hypoxia, without change in AP, increased the sequence and ramp gain by 31% and 65%, respectively. By contrast, the concomitant increase in resting RSNA (27%) and AP (14 mmHg) by glutamate infusion into the RVLM caused similar increases in the sequence and ramp gain (55% and 50%, respectively). The sequence gain, estimated during the nitroprusside-induced sympatho-activation (187%) and hypotension (-24 mmHg), was twice that in control conditions, at 36 ± 5 nu/mmHg.

The gain estimated by thesequence method is higher than the ramp estimate, possibly due to the dynamic nature of the spontaneous baroreflex. However, the sequence gain appears to be more readily influenced by the relative levels of RSNA and AP.

The effect of chronic rilmenidine on baroreflexes in 2kidney 1clip (2K1C) hypertensive rabbits.
SL Burke & GA Head.

We assessed the effects of chronic administration of the antihypertensive agent rilmenidine on heart rate (HR) and renal sympathetic nerve activity (RSNA) baroreflexes in conscious rabbits made hypertensive by renal clipping.

Rabbits were fitted with a clip on the right renal artery and two weeks later an osmotic minipump delivering rilmenidine was inserted subcutaneously. A recording electrode was implanted on the left renal nerve two weeks later. To produce RSNA and HR

baroreflex curves, phenylephrine was used to induce ramp rises in mean arterial pressure (MAP) and inflation of a perivascular vena caval cuff lowered MAP.

Five weeks after clipping, MAP was 27% higher than the preclip level in saline treated rabbits but no different in the rilmenidine treated group. The HR baroreflex curve was shifted to the right and the hypertension and sensitivity were reduced by one third. Rilmenidine reversed these changes such that the HR baroreflex curve 5 weeks after clipping and after 3 weeks of treatment, was identical to that before clipping. Rilmenidine treatment attenuated the RSNA baroreflex to 35% less than that of saline treated animals. The curve was shifted to the left with the reversal in MAP. Resting RSNA was also reduced. Left ventricle/body weight ratios were 19% lower in rilmenidine treated rabbits.

Treatment with rilmenidine in 2K1C hypertensive rabbits completely normalizes blood pressure, prevents left ventricular hypertrophy and restores the HR baroreflex sensitivity.

Grants and other funding

Influence of ICV aminopeptidase A inhibitors, on blood pressure and heart rate in conscious spontaneously hypertensive rats.
G Head, Servier Laboratories, $54,545.

Influence of chronic Rilmenidine treatment on cardiac and renal baroreflexes and renal function in hypertensive rabbits.
G Head, Servier Laboratories, $121,500.

Chronic effects of NV04 and NV05 on blood pressure in spontaneous hypertensive rats.
G Head, Novogen, $102,000

Visiting Scientists

Dr Ben J A Janssen, Department of Pharmacology, Faculty of Medicine, University of Limburg, The Netherlands.

Dr EV Lukoshkova, National Cardiology Research Center, Moscow, Russia

Presentations

11th European Meeting on Hypertension, Milan, Italy

Satellite Symposium of the 11th European Meeting on Hypertension, "Heart rate variability and baroreflex sensitivity: Methodological, Physiological and Clinical Aspects", Verbania, Italy

The 34th IUPS Congress in Christchurch, New Zealand

A Satellite of the 34th IUPS Congress 2001 "Central mechanisms of cardiovascular control - integrative, cellular and molecular aspects" in Sydney, Australia

A Satellite of the 34th IUPS Congress 2001 in Queenstown New Zealand

International Telemetry User Group in Queenstown, New Zealand

3rd France Australia Meeting on Hypertension, Porticcio, France

4th International Symposium on Vasoactive Peptides, Belo Horizonte, Brazil

2nd Australian User Telemetry Users Group Meeting, Melbourne, Australia

23 Annual Scientific Meeting of HBPRC of Australia, Melbourne, Australia

The Australian Neuroscience Society, Brisbane, Australia

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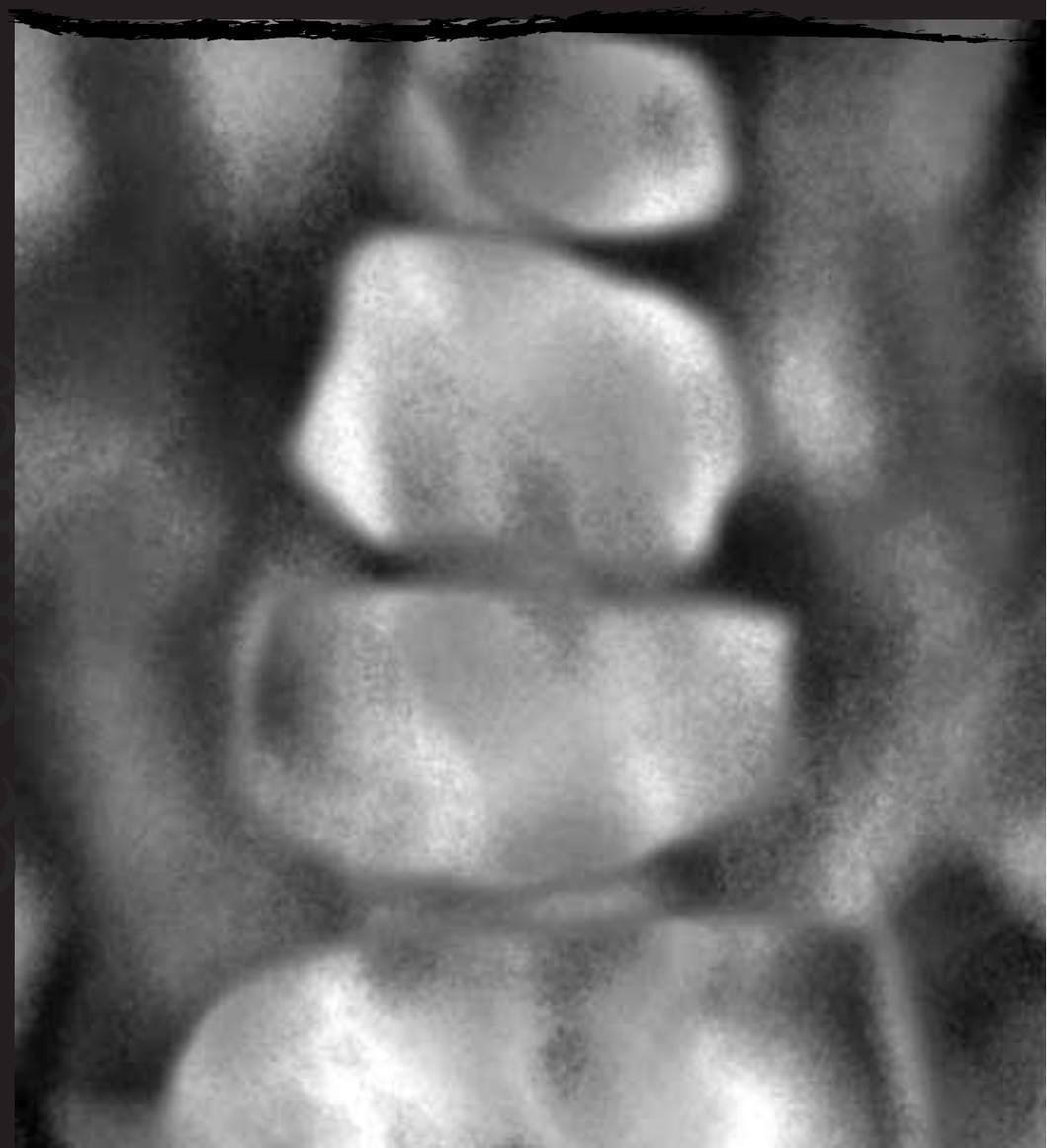
Associated Laboratories

... ABMU provides specific research platforms for clinical laboratories for invasive and non-invasive testing, clinical databases, clinical trial networks and a gene bank.



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Research projects

The CVD Prevention Unit is coordinating a number of national and international clinical trials.

2nd Australian National Blood Pressure (ANBP2) Study
ANBP2 is a large clinical outcome trial comparing two types of treatment for high blood pressure: ACE inhibition versus diuretic-based regimens. The study addresses important issues of managing hypertension in older people and has been undertaken as a joint venture project between the Commonwealth Government, the pharmaceutical industry (Merck, Sharp & Dohme) and the High Blood Pressure Research Council of Australia.

More than 6,000 hypertensive subjects have been monitored by more than 2,000 GPs over five years for cardiovascular events – heart attacks, strokes and other non-fatal outcomes. About 3,000 subjects have been involved in additional sub-studies of ANBP2, including the importance of left ventricular hypertrophy and ambulatory blood pressure monitoring in the management of hypertension in the long term.

OPERA Study
The OPERA study is an international, multi-centre, randomised, placebo-controlled trial of the treatment of borderline isolated systolic hypertension in older people using the novel agent, omapatrilat. Omapatrilat is effective for lowering blood pressure and may have particular advantages over other agents in managing systolic hypertension. Due to world-wide difficulties in recruiting subjects, OPERA was prematurely closed in November 2001

OCTAVE Study
The OCTAVE study is an international, multi-centre, randomised, placebo-controlled trial of the treatment of hypertension in older people using a novel agent, omapatrilat. We have recruited 125 subjects to OCTAVE.

ASCTS Database Project
The Victorian Division of the Australian Society of Cardio-thoracic Surgeons has appointed the Baker Medical Research Institute as the centre for Data Management and Analysis for a project to identify key performance indicators for cardiac surgical outcomes. A standard database had been developed for implementation at each public hospital in Victoria and risk-adjusted outcome models are under development to measure surgical performance. The aim is that the Victorian project will be extended on a national – and ultimately international – basis.

ONTARGET Study
The BMRI is the National Coordinating Centre for the ONTARGET trial which is a randomised trial of an angiotensin II antagonist, telmisartan, versus an Angiotensin Converting Enzyme (ACE)-inhibitor, ramipril, versus a combination of the two agents. The study aims to recruit 4,000 Australian patients of a total of 28,000 world-wide and follow their cardiovascular outcomes over five years.

Australia Heart Trial
The Australia Heart Trial was a pilot study, done in collaboration with the Centre of Molecular Biology and Medicine. It was a placebo-controlled, randomised trial to examine the effects of Co-enzyme Q10 in patients receiving statin therapy who reported pain and symptoms of muscle fatigue.

Meta-analysis of left ventricular hypertrophy
This project involved a systematic review of studies examining the effects of left ventricular hypertrophy (LVH) and regression on cardiovascular outcomes. We showed that LVH is associated with an approximate two-fold increased risk for all cause mortality and 2.3-fold increased risk for non-fatal and fatal cardiovascular events.

Ambulatory Blood Pressure Monitoring (ABPM) Study
Using a prospective clinical trial this project aims to determine the rate of morning rise in blood pressure in a large patient group and to identify predictors of early morning surge in BP in hypertensive versus normotensive people. Volunteer subjects presenting for clinical diagnosis of hypertension and those attending a community-based risk factor screening clinic will undergo 24 hour ABPM and provide a lifestyle and medical history. Subjects will be monitored annually for three years, after which all baseline measurements will be re-assessed. We will use multivariate analysis of variance to determine predictors of the rate of rise and fall of BP and heart rate in normotensive and hypertensive patients.

Diabetes detection in General Practice
This project involved analysis of data from a large diabetes screening study in general practice to determine the impact of the change in diagnostic criteria for classification of diabetes. We found that the WHO criteria were preferable to the US criteria as the latter underestimated the prevalence of undiagnosed diabetes by almost half.

Secondary prevention of stroke in general practice
This is a collaborative project with Royal Melbourne Hospital exploring the benefits of a shared care approach to the management of patients post-stroke. The project is in its first year and 80 subjects have been enrolled.

Secondary prevention of cardiovascular disease in general practice (Sentinel)
The aim of this project is to increase the use of evidence-based management of cardiovascular disease in general practice. Across Australia, 1400 GPs have enrolled in this project. Each will review the records of 30 subjects. A computer-assisted decision support program will provide the GP and patient with an evidence-based approach to management and the subjects will be reviewed at three months. The target is to increase the proportion of subjects achieving target risk factor levels.

Primary prevention of cardiovascular disease in general practice (cvTRACplus / Heart Care)
To assist GPs identify and manage risk factors for cardiovascular disease in general practice, we have developed computer-based data collection and reporting programs. Over 60,000 patients will be involved in this project over the next two years. The project aims to improve the control and management of cardiovascular risk factors.

Grants and other funding

Secondary prevention of stroke in general practice
J Joubert, CM Reid, D Ruth, P Joubert and S Davis, Commonwealth Dept of Health and Aged Care, 2000-2002, $42,000

Analysis of perindopril in the treatment of hypertension
G Jennings and C M Reid, Servier Laboratories, $20,000

OPERA Study
CI Johnston and CM Reid, BristolMyerSquibb, $625,000

OCTAVE Study
CI Johnston and CM Reid, BristolMyerSquibb, $428,000

cvTRAC / Heartcare- risk factor management in general practice
CM Reid and M Rockell, AMRAD Pharmaceuticals and Merck, Sharp & Dohme, $55,000

Sentinel
CM Reid, BristolMyerSquibb, $110,000

Presentations

5th International Society of Preventive Cardiology, Osaka, Japan

11th Scientific Meeting of the European Society of Hypertension, Milan, Italy

7th WHO-ISH Blood Pressure Collaboration Meeting, Cambridge, UK

1st World Heart Federation Global Conference on Cardiovascular Clinical Trials, Hong Kong (Session Chair)

Scientific Meeting of the Cardiac Society of Australia and New Zealand, Auckland

High Blood Pressure Research Council of Australia, Melbourne

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The ABMU is the clinical research at the Baker. A successful NHMRC Centre of Clinical Excellence in Hospital-based Research Grant to the ABMU supported translation of research findings to clinical practice.

Specific research findings are reported elsewhere in individual laboratory reports from Cardiovascular Nutrition, Cell Biology, Clinical Physiology, Experimental Cardiology, Human Neurotransmitter Research, Molecular Neurocardiology and Vascular Pharmacology.
ABMU provides specific research platforms for these activities. These include clinical laboratories for invasive and non-invasive testing, clinical databases, clinical trial networks and a gene bank.

Risk Reduction Clinic

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Di Wilson SRN

ADMINISTRATION
Amanda Coates BA Mon (Menopause Clinic)

The Risk Reduction Clinic performs free screening to members of the community for risk factors related to diseases of the heart and circulation.
The approach to screening is to apply simple and cost-effective tests, linked to lifestyle, that are of proven usefulness. We measure cholesterol and triglycerides and obtain information from a lifestyle questionnaire. Where necessary, the initial contact with the Risk Clinic may be followed up by medical intervention.

A close link exists between the Risk Clinic and the ABMU research interests in prevention of cardiovascular disease, nutrition and exercise.
Staff at the Risk Clinic are involved in acquiring samples for the Gene Bank and a broad range of research studies in addition to their critical role of recruiting subjects for ABMU studies.

Research continued into finding better methods of defining risk in healthy subjects. In collaboration with Dr David Torpie of the University of Queensland a study of the genetic basis of chronic fatigue syndrome was recently completed.
The clinic provides a base for the Menopause Clinic and also for nutrition studies performed by the Cardiovascular Nutrition Laboratory.

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