Health means the world to us.

BAKER HEART RESEARCH INSTITUTE

RESEARCH REPORT 2004

PROFESSOR GARRY JENNINGS

This research report is a compilation from our scientific laboratories. The intention is to provide a volume that may be of use to our scientific colleagues who are interested in the scientific laboratories of the Institute and their progress. It is also a record of some of the intermediate measures of scientific productivity such as publications, grants, invited lectures and other indicators of the standing of our scientists in their respective fields. As such it may prove impenetrable in parts to the general reader. A more accessible account of the science of the Institute is included in our annual report, to which these readers, looking for a less technical and detailed account are referred.

Scientific progress requires creativity, industry and skill from the talented scientists who contributed to this report. However they will be the first to admit that it utterly depends on support from mentors, colleagues, administration, management and the services side of the Institute, collaborators, fundraisers and grant giving agencies. On behalf of our staff I would like to thank all of those who have played a part.

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Director
Baker Heart Research Institute

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HEART - CARDIOLOGY DIVISION

David M Kaye

The Cardiology division brings researchers with a diverse range of skills together to study the heart in health and in disease. The division, headed by Wynn Professor David Kaye, spans molecular cardiology labs who conduct basic research into the 'nuts and bolts' of how the heart works, through to clinical researchers working with patients awaiting heart transplant.

Key molecular mechanisms of hypertension, heart cell death and cardiac hypertrophy are being investigated by Dr Wally Thomas and Associate Professor Liz Woodcock.

At a more clinical level, Dr Jaye Chin-Dusting investigates changes occurring in aging arteries, work of special relevance to Australia's aging population. Dr Sal Pepe investigates novel surgical techniques, and Dr John Power conducts applied cardiovascular research into devices to assist with heart failure. Highlights this year include the first patient implants of a percutaneous mitral annuloplasty device, a device to fix leaky mitral valves, and the formation of a Baker start-up company, V-Kardia, to develop a device to deliver targeted gene therapy treatments to the heart.

Some members of the division cross traditional scientific boundaries, such as Dr Assam El-Osta, whose human epigenetics laboratory has ongoing projects with other division members to look at the role of structural changes to genes in a number of processes in health and disease. Likewise, the experience with small animal models and microsurgical skills of Dr Xiao-Jun Du are being applied to projects across the entire division and the larger institute as a whole. Dr Rebecca Ritchie also acts to connect the cardiology division to other researchers within the institute with her work looking into the effects of diabetes on the muscle of the heart.

The Cardiology division brings together such diverse groups in order to address the 'big issues' in heart research - diseases such as heart failure, arrhythmia and heart attack, which require a major public health focus and are a leading cause of years lost to morbidity and mortality in our society. The Cardiology division harnesses the talents of each of its members to work toward the larger goal of preventing cardiovascular disease in Australia and around the world.

To this end, the Cardiology division has benefited greatly from the very generous support of Professor Victor Wynn and the Atherosclerosis Research Trust (based in the UK). Such support has allowed considerable progress to be made in our extensive program of research directed at understanding the metabolic basis for heart failure and its underlying causes.

WYNN DEPARTMENT OF METABOLIC CARDIOLOGY

David M Kaye

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Christine Goh - Honours
Greta Meredith - Honours
Helen Rancie - Honours

Research Projects

Oxidized LDL reduces the uptake rate and intracellular content of arginine via depletion of cellular membrane cholesterol

Wei-Zheng Zhang, David Kaye

The atherogenic effects of oxidized LDL (oxLDL) have been found, at least in part, to be the result effects on endothelial-derived nitric oxide (NO). OxLDL causes an efflux of caveolar cholesterol out of the cell membrane resulting in the displacement of eNOS from caveolae thereby impairing nitric oxide synthesis. Cationic amino acid transporter 1 (CAT1) is closely associated with eNOS and located in membrane caveolae. In this study we hypothesized that oxLDL may affect arginine transportation through altering CAT1 membrane expression

Initial recent studies demonstrated that oxLDL reduces cellular arginine uptake and arginine intracellular concentration together with reduced NO generation. Further, we have now studied the influence of modulating membrane cholesterol, using beta-cyclodextrin. This agent reduces the cholesterol content of cell membrane, and led to decreased CAT1 activity. This process may be an important mechanism in the early pathogenesis of atherosclerosis. These studies show a significant effect of LDL on the metabolism of arginine by endothelial cells including a reduction in arginine transport.

Effects of cigarette smoke on arginine metabolism

Wei-Zheng Zhang

Cigarette smoke is widely recognized as a risk factor for endothelial dysfunction and cardiovascular disease, however the precise mechanism for this effect is unclear. In the present in vivo, in vitro and clinical studies we demonstrate that smoking substantially attenuates the activity of L-arginine:nitric oxide synthase pathway via multiple mechanisms. In particular cigarette smoke reduces the contents of L-arginine, citrulline and NOHA, while increases that of ADMA (a cardiovascular risk factor). Investigating on molecular mechanism, we reveal that those effects are caused, at least in part, by chemically modification of the active molecules (eg. arginine transporter and key enzymes) with smoke constitutes. These findings provide (1) a further insight into the mechanism responsible for the deleterious effects of cigarette smoking on endothelial function and (2) the basis for a link between smoking and elevated ADMA in the context of cardiovascular disease.

Molecular biological production of active DDAH2 and its modifications

Wei-Zheng Zhang and Zhiyong Yang

ADMA is a naturally occurring methylated arginine that inhibits the synthesis of NO from L-arginine to impair endothelium-dependent vasodilation. It is actively metabolised by DDAH. Thus variation in DDAH activity may be an important mechanism underlying variation in ADMA concentrations, either locally within endothelial cells or systemically.

It has been demonstrated DDAH1 can be actively modified in vivo upon specific cys-s-nitrosylation following with a reduced activity. The DDAH2 isoform is highly expressed in endothelium and heart, and therefore regulation of its activity may be important for cardiovascular system. We have successfully cloned human DDAH2 and transformed it into E. coli. Screening for an expression system is currently underway, with the aim of generating active DDAH2 molecules. Upon obtaining active DDAH2, the effects of chemical modifications on its activity will be assessed. Protection of DDAH2 against nitrosylation will also be investigated.

Mitochondrial Arginine Transport & Oxidative Stress

Belinda Smirk, David Kaye

A reliable method for the isolation of intact mitochondria has been established for the measurement of arginine uptake and superoxide generation. We have found that mitochondria exhibit robust uptake of L-arginine. In conjunction, evidence is accumulating that arginine supply to the mitochondria could influence superoxide generation.

ADMA levels in diabetic subjects: implications for outcomes

Nagesh Anavekar

Diabetes is characteristically associated with heightened cardiovascular risk. We are attempting to develop/identify biomarkers that may prove to be useful predictors for diabetics at risk. To do this we are currently performing measuring ADMA levels in a large cohort of diabetic subjects.

Modulation of Arginine Transport by Insulin

Claire Gologly, David Kaye

The effects of intra-arterial delivery of insulin in normal volunteers will be analysed by HPLC. Once these data are available we will commence studies of the effect of intra-arterial insulin in hypertensive and diabetic subjects.

Establishment of a mCAT-1 transgenic mouse

Zhiyong Yang and David Kaye

Our previous studies have pointed to a potential genetic link between essential hypertension and abnormalities of arginine transport. Arginine transport by endothelial cells is predominantly mediated by the system y+ carriers. Among them, CAT-1 is the predominant transport system in endothelial cells and expresses almost ubiquitously in different tissues and cell types although its expression level varies considerably. We now have a well established colony of mice with endothelial specific overexpression of CAT1. Planned and ongoing studies include the isolation of aortic endothelial cells for explanted aortae; studies of endothelial function in the organ bath and functional studies of blood pressure control and susceptibility to atherosclerosis in intact animals.

Polymorphisms of the human CAT-1 gene

Zhiyong Yang and David Kaye

We have verified 12 polymorphisms around human CAT-1 gene. We will genotype these polymorphisms to detect disease association, as well as conducting real time RT PCR and RNase protection assays to compare mRNA expression levels of different alleles (GG, GA, AA); "modified RNase T1 selection assays" to compare mRNA stability; and gel shift assays to reveal any interaction with nuclear proteins. Construction is planned of human CAT-1-EGFP fusion genes containing different allele of 3' UTR polymorphism to study subcellular localisation of the fusion protein.

Construction of cDNA libraries from EaHy926 and HeLa cells

Zhiyong Yang

We have made RACE-ready cDNA libraries with HeLa cells and human endothelial cell line EaHy926. We have used these libraries to clone full-length human DDAH II gene and will be studying gene regulation and its relationship with arginine metabolism.

HEART FAILURE RESEARCH

Regulation of BNP expression in cardiomyocytes

Carla Enriquez and David Kaye

Recent research has clearly shown that the plasma levels of BNP rise in patients with congestive heart failure. Moreover, the level of BNP has been shown to be a useful predictor of outcome and the presence (or absence) of heart failure. At a cellular level, it has been proposed that myocardial stretch is the key stimulus for the expression and release of BNP. While this mechanism is relatively well understood, the precise cascade of molecular events that trigger BNP expression are poorly understood. We have commenced a series of studies to elucidate these mechanisms using Chromatin Immunoprecipiation assays. This technique will allow us to identify factors which interact with the BNP promoter. In this project we have exposed neonatal rat cardiac myocytes to control or stretched conditions. Stretch causes a significant increase in BNP expression. We are further investigating new evidence that the BNP increases its association with the protein alpha acetyl H4.

Generating a recombinant adenovirus containing the Norepinephrine Transporter

Tanneale Marshall, David Kaye

Heart failure is characterised by an increased activity in the sympathetic nervous system (SNS) to compensate for a reduction in cardiac output and to maintain tissue perfusion. Norepinephrine (NE), a neurotransmitter, is involved in signalling to increase heart rate and myocardial contractility. However, increased SNS activity commonly results in excess NE remaining within the myocardium. Reduced NE clearance is cardiotoxic and leads to myocyte death and attenuates heart failure. Recombinant adenoviruses provide a versatile system for gene delivery and transfer to cells in vitro and in vivo. We have now generated an adenovirus

containing the NET (noradrenaline transporter) gene. Our studies, for the first time, have shown that cardiac myocytes infected with adenoviruses expressing NET do indeed demonstrate noradrenaline uptake. Our studies will now address the possibility that myocytes expressing NET will be resistant to the deleterious effects of catecholamine overexpression (eg preventing apoptosis). If proven we will then proceed to in-vivo studies of NET gene transfer in animals with heart failure.

Establishing inducible cardia-specific transgenesis of nerve growth factor (NGF) using attenuated myosin heavy chain promoter

Zhiyong Yang and David Kaye

In previous studies we determined that nerve growth factor was deficient in the failing human and animal heart. To address the potential for NGF to reverse the heart failure phenotype we are constructing a gene that will produce regulated expression of NGF in the heart. We have made "responder" DNA construct with modified alpha-MyHC and rat NGF cDNA. We will test NGF gene expression in mammalian cells before trying to establish inducible transgenic mouse lines.

MOLECULAR AND CELLULAR CARDIAC PHARMACOLOGY

New targets for preventing cardiac hypertrophy

Rebecca Ritchie, Anh Cao

Cardiac hypertrophy is a consequence of long term, untreated hypertension and is linked to the progression of heart failure. Increased formation of reactive oxygen species is now emerging as an important factor in this disease progression. We have recently demonstrated that the neurohormones angiotensin II (Ang II) and endothelin-1 (ET1) induces increases in cardiomyocyte size and expression of the hypertrophic genes c-fos and b-myosin heavy chain (b-MHC). These hypertrophic actions are accompanied by increased generation of the reactive oxygen species superoxide. Furthermore, the cardiac hormone atrial natriuretic peptide (ANP) blocks the increase in all three markers of cardiomyocyte hypertrophy, as well as significantly attenuating Ang II-and ET1-induced superoxide generation. Both the antihypertrophic actions and antioxidant actions of ANP in cardiomyocytes were mimicked by the superoxide spintrap tempol. Neither ANP nor tempol influenced markers of hypertrophy or superoxide generation when studied alone. These findings clearly demonstrate that the antihypertrophic actions of atrial natriuretic peptide (ANP) are associated, at least in part, with an antioxidant action. The next step in these studies is to explore whether other cyclic GMP-elevating agents including B-type natriuretic peptide (BNP) and nitroxyl anion (NO-), also elicit antihypertrophic actions via antioxidant mechanisms. Inhibition of reactive oxygen species, such as with a nonpeptide natriuretic peptide receptor agonist, may be a potential target for pharmacological prevention of hypertrophy.

Influence of sex hormones on cardiac hypertrophy

Rebecca Ritchie, Ruchi Patel

The effects of the female sex hormone oestrogen on cardiovascular outcomes are presently controversial and poorly understood. Premenopausal women have lower incidence of cardiovascular events than their age-matched male counterparts, a protection lost post-menopause. Oestrogen supplementation in postmenopausal women however may increase the risk of cardiac events. In collaboration with A/Prof Igor Wendt (Dept of Physiology, Monash University), we are exploring the effects of sex hormones on cardiac hypertrophy. The male sex hormone testosterone has pro-hypertrophic actions in neonatal rat cardiomyocytes isolated from pups of mixed gender. Cardiomyocytes isolated from adult male rats elicit the expected hypertrophic responses (increased de novo protein synthesis, etc) to neurohormones including Ang II, ET1 and phenylephrine(PE); myocytes from age-matched adult female rats however do not show an hypertrophic response to these stimuli. Conversely, hypertrophic responses in cardiomyocytes isolated from either gender of neonatal rat pups are equally robust. Recent results indicate that pre-treatment of male neonatal rat cardiomyocytes with oestrogen for 24h abolishes the hypertrophic response to neurohormones. A similar benefit is observed when oestrogen and PE are studied in combination. This protective action of oestrogen is however absent in female neonatal rat cardiomyocytes. We propose to compare these findings obtained to date with myocytes from adult rats post gonadectomy and/or chronic sex hormone (oestrogen or testosterone) administration in vivo. Optimal clinical management of cardiac hypertrophy in humans may require sex-specific approaches.

Role of reactive oxygen species in diabetic cardiac disease

Rebecca Ritchie, Anh Cao

Diabetic cardiac disease is a distinct and common entity, characterised by cardiac hypertrophy (enlargement), myocardial fibrosis (stiffening) and cardiac dysfunction (impaired pumping). Reactive oxygen species are now

considered to mediate many of the vascular complications of diabetes, but the extent to which they play a role in cardiac complications of the disease is yet to be elucidated. We have shown in a rat model of type 1 (insulin-dependent) diabetes eight weeks post induction of diabetes with streptozotocin, that cardiac generation of superoxide is increased 2-3-fold. This setting of cardiac oxidative stress is accompanied by increased heart to body weight ratio, and cardiac upregulation of both ANP and the related B-type natriuretic peptide (BNP), as well as c-fos and b-myosin heavy chain (b-MHC) gene expression, all indicative of cardiac hypertrophy. There is also histological evidence of increased cardiac fibrosis. No evidence of hypertension is detected. Moreover, in collaboration with Drs Xiao-Jun Du and Helen Kiriazis (Experimental Cardiology, Baker Institute) recent echocardiographic and cardiac catheterisation analysis indicates that our model of diabetic cardiomyopathy also includes marked impairment of diastolic function (reduced E/A ratio, LV-dP/dtmin), with some suggestion of declining systolic function (velocity of circumferential fibre shortening, LV+dP/dtmax). Administration of either a subpressor dose of the cardiac hormone ANP, or insulin, for the final 4 weeks of diabetes, reverses the cardiac hypertrophy and generation of superoxide. Some evidence for protection with antioxidant or ACE inhibitor administration is also seen. The next step of these studies is determining whether cardiac-specific gene therapy, with overexpression of the mitochondrial isoform of superoxide dismutase, MnSOD, also preserves cardiac function and structure in the diabetic heart. Therapeutic strategies targeting oxidative stress in the heart may have potential utility in the clinical management of diabetic heart disease.

Role of reactive oxygen species in insulin-resistant cardiac disease

Rebecca Ritchie, Greta Meredith, Anh Cao

Insulin resistance is regarded as an important defect that ultimately contributes to the development of type 2 diabetes). The GLUT4-deficient mouse, a model of insulin resistant cardiomyopathy, exhibits marked cardiac hypertrophy (increased heart to body weight ratio and cardiac expression of b-MHC) and fibrosis (cardiac gene expression of pro-collagen III). Cardiac expression of both Nox1 (a subunit of NADPH oxidase, an important source of superoxide in the heart) and its homologue gp91phox are also increased; cardiac generation of superoxide is however not different. Treatment of these mice with the antioxidant tempol in vivo over a 4 week period reduces both hypertrophy and fibrosis indicators. In collaboration with Dr Lea Delbridge, Dept of Physiology, University of Melbourne, we have compared effects of a high sucrose diet and a high fructose diet with matched carbohydrate feeding over 6 weeks in mice in vivo. The diets are matched for kilojoule content. No changes in blood glucose (non-fasted), blood pressure, weight gain, or heart to body weight ratios were observed with either sugar. Both cardiac superoxide generation and cardiac expression of b-MHC were however elevated in the mice fed a high fructose diet. We speculate that elevated cardiac generation of reactive oxygen species may precede cardiac hypertrophy and other cardiac abnormalities associated with insulin resistance. Thus, therapeutic strategies targeting oxidative stress in the heart may have potential utility in the clinical management of insulin-resistant heart disease.

New approaches to protecting the heart from ischaemia-reperfusion injury

Anh Cao, Christine Goh, Rebecca Ritchie

Ischaemic heart disease is a major public health problem in Australia. New therapeutic strategies for the treatment of myocardial ischaemia in humans are therefore highly desirable. We have recently found that a small protein derived from the endogenous anti-inflammatory hormone annexin-1 protects isolated cardiomyocytes from ischaemic injury. Protection with annexin-1 is evident whether metabolic (lactic acid, low pH, inhibition of respiration) or oxygen-deprivation (hypoxia) means of simulating ischaemia are used. Recently we have demonstrated that the increase in cardiomyocyte superoxide generation seen on reoxygenation is roughly halved with annexin-1 treatment. Moreover, in collaboration with Dr Sal Pepe (Cardiac and Metabolism Surgery, Baker Institute), we have taken these studies into the whole heart. Isolated Langendorff-perfused rat hearts subjected to 30mins global, no-flow ischaemia followed by 20mins reperfusion show marked cellular injury (indicated by release of LDH and CK into the effluent), and impaired recovery of cardiac contractile function (LV systolic pressure and LV +dP/dtmax). However administration of annexin-1 just prior to ischaemia markedly prevents the cellular injury and the rate and extent of recovery of cardiac function. In a similar series of studies in collaboration with A/Prof Owen Woodman (Dept of Pharmacology, University of Melbourne), we have also demonstrated powerful cardioprotective actions of resveratrol, in adult rat cardiac myocytes subjected to hypoxia/reoxygenation. Protection is evident at the level of cellular injury

(LDH release) and cell viability (trypan blue); these actions are mimicked by the antioxidant tempol. Opening of potassium channels in cardiomyocytes (both ATP-sensitive KATP and calcium-dependent KCa channels) are implicated in reseveratrol cardioprotection The increase in cardiomyocyte superoxide generation seen on reoxygenation is completely abolished with resveratrol treatment. Moreover, when resveratrol is added to the perfusion buffer in isolated Langendorff-perfused rat hearts, release of LDH and loss of recovery of contractile function are largely prevented by resveratrol.

Differences between physiological and pathological cardiac hypertrophy offer new strategies for treating heart failure. JR McMullen.
Growth of the heart can be induced by physiological stimuli (eg postnatal development, chronic exercise training) or pathological stimuli (eg pressure or volume overload). Cardiac hypertrophy induced by exercise training, commonly referred to as "the athlete's heart", is generally considered a benign or beneficial adaptation. In contrast, when disease causes pressure or volume overload (e.g., hypertension, valvular disorders) of the heart, the resulting cardiac hypertrophy is initially a compensatory response to the increased load. However, function in the hypertrophied heart eventually decompensates, leading to left ventricle dilation and heart failure. Reasons for this paradox are still under investigation. One possible explanation is that pathological and physiological hypertrophy are distinct processes mediated by distinct signalling pathways. The mechanistic process which allows the heart to enlarge in response to physiological stimuli while maintaining normal or enhanced function is of clinical relevance, as one potential therapeutic strategy would be to inhibit the pathological growth process while augmenting the physiological growth process.
The goal of this research is to determine whether activation of a gene thought to be critical for exercise-induced cardiac hypertrophy i.e. the p110a isoform of phosphoinositide 3-kinase (PI3K), could be a potential tool for augmenting physiological growth and improving cardiac function of the failing diseased heart, to examine the underlying mechanisms responsible, and to identify novel signalling molecules important for the induction of physiological cardiac hypertrophy. The identification of signalling molecules and cascades which could be utilised to improve cardiac function and myocyte survival will be an important addition to the standard therapy for patients with heart failure.

Grants and other Funding

- Atherosclerosis Research Trust, Support for Wynn Department of Metabolic Cardiology.

- BIF Grant, Role of novel guanidino compounds in cardiovascular disease.

- NHMRC Program Grant, Heart Failure and its Antecedents : Pathophysiology, Prevention and Treatment, D Kaye Co-Chief Investigation.

- NHMRC Special Initiatives Grant, Mechanisms of cardiac cachexia, D Kaye & J Power.

- NHMRC/National Heart Foundation Career Development Award, Differences between physiological and pathological cardiac hypertrophy offer new strategies for the treatment of heart failure, J McMullen.

Commercially Funded Research Activities

- Cardiac Dimensions Inc, Cardiac Device Therapy in CHF. D Kaye and J Power.

Presentations

- CSANZ Annual Scientific Meeting, Schlaud, M Parnell, W Zhang, D Kaye, Impaired L-Arginine uptake in essential hypertension is not due to ADMA.

- W Zhang, D Kaye, Effects of cigarette smoke on arginine metabolism.

- D Kaye, Update on ventricular assist devices. (workshop)

- D Kaye, M Bryne, Alferness, Reuter, J Power, Percutaneous mitral annular reduction.

- The XVII World Congress of the International Society for Heart Research, Brisbane. D Kaye, CHF Neurobiology.

- Scientific sessions of the American Heart Association, USA., W Zhang, D Kaye, Effects of cigarette smoke on arginine metabolism.

EXPERIMENTAL CARDIOLOGY LABORATORY

Xiao-Jun Du

Anthony M Dart

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Research Projects

Predictive value of echocardiographic parameters in post-infarct ventricular rupture

XJ Du, Z Ming, XM Gao, H Kiriazis, AM Dart

Rupture of free wall of the left ventricle (LV) occurs in patients with acute myocardial infarct (AMI) leading to sudden death. Prediction of rupture event remains difficult due to lack of reliable biophysiological markers. Using a mouse model of post-AMI rupture, we examined whether echocardiography prior to the onset of rupture could reveal difference in animals with and without rupture. Coronary artery occlusion was performed in male 129sv mice and echocardiography was conducted under avertine anesthesia 48 hours after AMI. Animals were monitored and autopsy was performed to identify rupture death and infarct size (IS). The rupture-free mice were killed at day 7 and divided into 2 groups according to infarct size (IS). From LV short- and long-axis loops, M-mode LV images and color Doppler aortic flow, we measured LV diastolic and systolic volumes (EDV, EDV) or dimensions (LVDd, LVDs), fractional shortening (FS), and ejection fraction (EF). Of 97 mice with AMI, 60 (62%) died of rupture during 2 to 6 days. Compared with surviving mice with comparable IS (47±10 vs 46±8%, mean±SD), ruptured mice had greater ESV (28±12 vs. 23±8ml, $P<0.01$) and LVDs (3.6±0.7 vs. 3.1±0.5 mm, $P<0.01$), suppressed EF (40±11 vs. 45±10%, $P<0.05$) and FS (14±10 vs. 24±11%, $P<0.01$), more severe regional LV remodeling and wall thinning ($P<0.01$). These indices also have predictive value for rupture. In conclusion, development of rupture in the murine model is preceded by more severe LV remodeling and systolic dysfunction and that these changes are detectable by echocardiography.

Ventricular rupture in the infracted heart is due to damage of the existing myocardial collagen network

XM Gao, CS Samuel, H Kiriazis, KF Lu, AM Dart, XJ Du

Ventricular wall rupture occurs in mice with acute myocardial infarct (AMI) and therefore is a suitable model for rupture. We studied the importance of extracellular matrix (ECM) collagen in the risk of rupture in mice with AMI by coronary artery occlusion. Rupture occurred during days 2-6 and the incidence varied between strains from 25-35% in C57B6 to 50~70% (129sv). Collagen content in the infarcted tissue, as determined by hydroxyproline assay, was unchanged at Day-2 but increased by 60% at Day-4 after AMI, when rupture occurred. We then determined tension-to-rupture (TTR) in vitro using ventricular rings from hearts with AMI for 4 days. There was a 60% reduction in TTR of infarcted compared to that of non-infarcted rings either from the same hearts or from sham-operated mice, indicating a significant reduction in muscle tensile strength. Our previous studies showed that mice with cardiac overexpression of b2-adrenoceptors had a reduced risk of rupture. Measurement of TTR in the transgenic mice revealed a 30-40% higher TTR than that of non-transgenic controls with or without AMI, a change in keeping with a 40% increase in myocardial collagen content (2.63±0.18 vs 1.61±0.07 mg/mg d.w., $p<0.01$, $n=6$ each). To compare these findings with a non-rupture rodent, we also similarly studied SD rats. Collagen content in the heart was 50% higher in rats than that in mice (2.32±0.04 vs 1.38±0.04 mg/mg d.w., $p<0.01$, $n=6$ each). In rat hearts with AMI for 4-days, TTR was unchanged versus that of non-infarcted ventricular rings. We then determined abundance and activity of type 2 and type 9 matrix

metalloprotenases (MMP) in infarcted tissues at Day-4 after MI by gelatine zymography. The major form of MMP that increased in response to AMI was MMP9 in the mouse but MMP2 in the rat and the ratio of MMP9:MMP2 in the infarcted tissues was 6 for the mouse and 0.6 for the rat. In conclusion, mouse ventricle with AMI has significant reduction in tensile strength which is related to ECM collagen content of. Following AMI, activation of MMP9 is likely to degrade collagen and lowers mechanical strength leading to rupture. Differences in the mouse and rat in subtypes of MMPs and collagen content indicate the importance of collagen damage in rupture pathogenesis.

Sex difference in the risk of post-infarct left ventricular rupture

Lu F, Gao XM, Kiriazis H, AM Dart, XJ Du

Cardiac rupture is a fatal complication of acute myocardial infarction (AMI). It has been reported that male mice had significantly higher incidence of cardiac rupture during the acute phase of AMI. Matrix metalloproteinases (MMPs) have been suggested to play important roles in healing and remodeling process post AMI. We hypothesized that the sex difference in the incidence of rupture after AMI in mice is attributed to sex difference in healing and remodeling process, especially MMPs activity. AMI was induced by coronary artery ligation in 129SV mice of both genders (4-5 months) and sham-operated animals served as controls. MMP-2 and –9 activities in left ventricular were measured by zymography. By day 4 after AMI, the accumulative incidence of rupture was significantly higher in male than female (64%, 16/25 versus 21.1%, 7/33, P<0.01). In AMI, latent-MMP-9 level in infarcted zone was found to increase moderately at day 2 and dramatically at day 4 (approximately 20 fold versus sham-operated). Such increase was more evident in male than female (P<0.05). In contrast, latent-MMP-2 level was moderately up-regulated in AMI (approximately 2 fold increase versus sham-operated), and female mice had higher pro-MMP-2 than male (P<0.05). Both active MMP-9 and –2 were at low levels in AMI, and active MMP-9 was slightly higher in male than female. Our results suggest that increment in MMP-9 content and activity in the infarcted myocardium is more pronounced in male than in female animals, which may contribute to a higher incidence of rupture in male than female mice. Thus, MMP-9 could be a target for interventions aimed at preventing post-infarct rupture.

Histone methylation and chromatin remodeling are epigenetic components responsible for mycardial gene profiles in the hypertrophied and failing heart

XJ Du, S Moore, H Kn, XM Gao, H Kiriazis, A El-Osta

Histone modifications remodel chromatin structure and transcription activity. Hypertrophied and failing myocardium are characterized by profound changes in gene transcription and molecular remodeling and it was not known whether an epigenetic program is involved in myocardial gene regulation during heart failure. We studied acetylation/ deacetylation or methylation states of histone tails and correlated these marks with transcriptional state of selected genes in hypertrophied and failing hearts of C57B6 mice with transverse aorta constriction (TAC) for 16-weeks. Mice with TAC developed severe left ventricular hypertrophy (179±11 vs 95±4 mg), chamber dilatation (4.8±0.3 vs 3.4±0.1 mm) and failure (fractional shortening: 22±3 vs 36±2%, all P<0.01 vs sham-operated group, SHAM). Real-time RT-PCR showed enhanced (b-MHC, ANF) or suppressed (a-MHC, SERCA2, b1AR) expression. Soluble chromatin fractions derived from cross-linked SHAM and TAC hearts were immunoprecipitated with antibodies against acetyl-H3, acetyl-H4, histone deacetylase 1 (HDAC1), or histone H3-K4 methylation using specific chromatin immunoprecipitation (ChIP) assays and real time-PCR to determine particular promoter sites. Chromatin from active gene sets were enriched in acetyl-H3 and H4 together with a concomitant release of HDAC1 in TAC vs. SHAM hearts, and the opposite occurred for the down-regulated gene sets. H3-K4 is known to activate transcription. We observed that the H3-K4 histone methyltransferase Set7 is mobilised onto transcriptionally competent genes (b-MHC and ANF) whereas H3-K9 histone methyltransferase Sue39 is dissociated. Interestingly, these epigenetic changes are reversible 6 weeks following removal of aorta banding. In summary, our results suggest that a coordinated and ordered response to hypertrophy and failing heart is the active remodeling of chromatin, and that mobilization of the epigenome is critical in regulating gene activity in heart disease

Reversal of established left ventricular hypertrophy by rapamycin

XM Gao, G Wong, H Kiriazis, Q Xu, S Moore, AM Dart, XJ Du

Left ventricular hypertrophy (LVH) in long-term is implicated as an important risk factor for cardiac morbidity and mortality. Therefore, reversal of cardiac hypertrophy is a major therapeutic target. The mammalian target of rapamycin (mTOR) is a component of

the insulin-phosphoinositide 3-kinase pathway, which is known to play a crucial role in determining cell and organ size. We have performed a study to induce LVH in mice by inducing transverse aorta constriction (TAC). Five weeks after TAC, when LVH is well established, some mice with TAC were treated with rapamycin, a specific inhibitor of mTOR for a period of 4 weeks. Echocardiography was performed to assess LV function and hypertrophic development. Compared with untreated TAC group, treatment with rapamycin for 4 weeks suppressed LVH by 54% judged by LV or heart weight normalized by body weight (3.4±0.2 vs. 4.0±0.2, P<0.01). Echocardiography showed a similar decrease in LV mass index and a significantly reduction in LV wall thickness (0.77±0.1 vs. 0.92±0.1 mm, P<0.05) while the contractile function was well maintained (LV fractional shortening 37±0.4 vs. 33±0.6, P =NS versus untreated TAC group). Further study is ongoing to explore the mechanism of LVH regression induced by rapamycin treatment. The positive outcome of this study may indicate an alternative pharmacological approach for treatment of hypertrophy.

Influence of a reproductive hormone relaxin on cardiomyocyte hypertrophy in vitro

S Moore, C Lo, XJ Du

Cardiac hypertrophy is one of the early events that characterise the clinical course of heart failure. Experimental and clinical studies strongly suggest that hypertrophy is a valid target for therapeutic intervention in treatment of heart failure. Recent studies have shown that relaxin, a reproductive hormone, can reduce cardiac fibrosis via modulation of cardiac fibroblast (CF) proliferation, differentiation and collagen production. Since our demonstration of the anti-fibrotic action of relaxin in the heart, we have extended our research on the possible effect of relaxin on the myocardial hypertrophy considering that activated fibroblasts might facilitate the hypertrophy via cell-cell interactions. Cardiomyocyte (CM) and CF are prepared from neonate rats. They are challenged with hypertrophic inducers, phenylephrine (PE) or Angiotensin II (AII), and set up as three groups: CM only, CM with CF conditioned medium and CM-CF co-culture to fully explore direct and/or indirect anti-hypertrophic potential of Relaxin. Hypertrophy will be estimated by assay of protein/DNA ratio, expression of hypertrophic marker gene ANF, and CM size. Following 48hr treatment of relaxin, cells are harvested. Cell size, their protein to DNA ratio and hypertrophic maker genes are examined. Preliminary data indicates that Relaxin has no direct anti-hypertrophic effect on CM induced by PE or AII. Experiments of CM and CF interaction are ongoing. The outcome of this study will provide insight into potential clinical use of relaxin in treatment of cardiac hypertrophy and fibrosis.

Relaxin reverses cardiac fibrosis induced by hypertensive heart disease in rats

ED Lekgabe, XJ Du, H Kiriazis, C Zhao, Q Xu, S Moore, Y Su, RAD Bathgate, CS Samuel

Cardiac fibrosis is a key characteristic of the structural remodeling that appears in hypertensive heart disease. Our previous studies have demonstrated that a peptide hormone relaxinis anti-fibrotic in the diseased heart. Using the spontaneously hypertensive rat (SHR) and WKY controls, we studied the effects of relaxin on myocardial fibrosis. Cardiac functional parameters were assessed using echocardiography and catheterization. Collagen content in the heart was analysed by hydroxyproline assay, electrophoresis and quantitative histology. Cardiac hypertrophy was assessed by myocyte size and expression of hypertrophy-associated genes by real-time PCR, while zymography was used to determine MMP expression and Western blotting used to determine expression of__-smooth muscle actin, a marker for myofibroblasts. We found that the left ventricular (LV) myocardium of 9-10 month old SHRs contained excessive collagen, (p<0.01). Fibroblast differentiation into myofibroblasts and the collagen-degrading enzymes, matrix metalloproteinase (MMP)-2 and -9 were also significantly increased in the LV of SHRs (p<0.05). Relaxin was able to reduce collagen content in the LV (p<0.01), inhibit fibroblast differentiation (p<0.05) and MMP-2 expression (p<0.05). However, relaxin did not affect cardiac hypertrophy in this model. In conclusion, relaxin is an anti-fibrotic agent that may have therapeutic potential in hypertensive heart diseases.

Heart rate and the risk of heart failure in pressure-overload mice

H Kiriazis, XM Gao, D Kaye, AM Dart, XJ Du

An increased heart rate at rest has been identified as an independent contributor to the risk of heart failure in persons with hypertension. This points to potential therapeutic benefits of heart rate reduction in hypertension, however, this has not been directly tested. Using a mouse model of long-term pressure overload, surgically induced by banding the aorta, we firstly examined whether heart rate plays a role in the development of heart failure in mice which would provide a model to further investigate the potential benefits of

heart rate reduction. Mice were followed for 17 weeks post-surgery with heart rate assessed from periodic echocardiography examinations. Approximately, 15% of mice developed heart failure, evidenced by the presence of pleural effusion, left atrial thrombus and lung congestion (with increased lung mass). Mice that developed heart failure had consistently higher anaesthetised heart rates across 3-17 weeks post surgery. When heart rate was determined in conscious mice under stressful conditions (simply holding the animal), heart rate was significantly lower in mice that developed heart failure, implying a blunted response to stress. In addition, transgenic mice with a tachycardia phenotype had a much higher incidence (~70%) of heart failure post aortic banding surgery. Taken together, mice with induced pressure overload and a relatively higher heart rate have an increased incidence of developing heart failure. We are currently investigating the effects of heart rate reduction using a drug that selectively reduces heart rate in humans and laboratory animals. An improved prognosis in the drug treatment group would add support towards incorporating a reduction in heart rate as an important part of a treatment regime for hypertensive patients.

Effects of aging in mouse heart expressing constitutively active a1B-adrenergic receptors

H Kiriazis, X Feng, XM Gao, Y Su, S Moore, XJ Du

Cardiac-directed overexpression of wild-type a1B-adrenergic receptor (AR) (26-43 fold) results in dilated cardiomyopathy and premature death at 9 months (mo) of age and suppression of b1-AR signalling. To investigate whether this heart failure phenotype is due to chronic activation of the a1B-ARs, transgenic mice (Milano et al: PNAS 1994;91:10109-13) expressing constitutively active a1B-AR by 2-fold in the heart (TG) and their non-transgenic (NTG) littermates were non-invasively studied at 6, 9, 12 and 15 mo of age using M-mode and Doppler echocardiography. Fractional shortening (FS) was significantly increased in TG versus NTG mice (44±2% vs 35±1% at 6-months and 40±2% vs 35±1% at 12-months, both $p<0.05$). Notably, the ratio of left ventricular (LV) early and atrial filling flow velocities (E/A) was reduced (1.31±.06 vs 2.1±.11 at 6-months and 1.26±.05 vs 2.09±.19, both $p<0.01$) and the deceleration time (DT) of the E-wave was prolonged in TG mice. This LV diastolic dysfunction was evident at 6 mo of age and persisted at the advanced ages. LV mass, estimated via echocardiography and normalised for body mass (LVM/BM), was not significantly different between TG and NTG mice, a finding that was verified at autopsy. Catheterisation experiments at 15 mo revealed unchanged LV contractility at baseline and blunted responses to b-agonist stimulation for heart rate and –dP/dt in TGs. In summary, unlike cardiac overexpression of wild-type a1B-ARs, expression of constitutively active a1B-AR does not impart detrimental effects leading to premature death.

Role of b-adrenergic receptors in the development of cardiac hypertrophy and heart failure induced by pressure-overload

H Kiriazis, K Wang, XM Gao, M Gibbs, S Moore, XJ Du

Congestive heart failure is a growing problem in the public health system. A common feature of heart failure is an enhanced activity of the sympathetic nervous system, with the unrelenting stimulation of the cardiac b-adrenergic receptors (b-AR). Contribution of b-adrenergic receptor (b-AR) signaling pathway to the development and progression of cardiac hypertrophy and dysfunction remain partially understood. To investigate this further, we have surgically constricted the aorta in mice with deletion of both b1-AR and b2-AR and also the normal controls. Echocardiography was performed at different time points throughout the 12-week study period, representing acute, subacute and chronic phases of the disease. Normally a sustained pressure-overload on the heart leads to left ventricular hypertrophy and, eventually, heart failure. Preliminary findings suggest that the mice deficient with b1/2-AR have a much reduced hypertrophic response of the left ventricle (LV) to the increased workload (LV:body weight ratio: 2.8±0.1 vs 5.8±0.2 mg/g; heart:body weight ratio: 3.8±0.1 vs 7.2±0.2 mg/g, both $p<0.01$), despite the sham-operated knockouts had a lower LV:body and heart:body weight ratio (2.4±0.1 vs 3.1±0.1 mg/g, and 3.3±0.1 vs 4.1±0.1 mg/g). Interestingly, left ventricular function estimated by echocardiography is maintained. These findings strongly suggest that b-adrenergic signalling pathways are involved in hypertrophic growth under diseased conditions and that hypertrophy is not essential for the heart to compensate functionally for an increased afterload. On-going work will help clarify the contribution of b-adrenergic receptors in the development of heart disease.

Mouse Surgery/Cardiology Core Facility

We continue to provide collaborative support to Baker groups and external

collaborative groups for research projects using small animals. This year we have been performing small animal surgery and assessment of heart function and trained a number of internal researchers for techniques (Molecular Cardiology, Human Genetics, Cell Biology, Vascular Pharmacology). We have also trained 3 external researchers. Dr Kiriazis has been actively involved in the AMREP Animal Ethics Committee.

Grants and Funding

- National Heart Foundation of Australia 2003-2004, Post-infarct left ventricular rupture: model characterization, prediction and prevention. XJ Du, AM Dart

- National Heart Foundation of Australia 2004-2005, Genetic enhancement of ventricular function in infracted heart by a1A-AR overexpression. XJ Du, RM Graham, RA Hannan

- NHMRC Program grant and NHMRC Fellowships (AM Dart, XJ Du)

Commercially Funded Research Activities

- We completed a study with IngenKO on cardiac phenotype of newly generated knockout mice.

Presentations

- American Heart Association Scientific Sessions 2004, USA: XJ Du, Post-Infarct ventricular rupture is due to matrix collagen damage in mice.

- American Heart Association Scientific Sessions 2004, USA, XJ Du, Cardiac-restricted overexpression of the a1A-adrenergic receptor preserves contractile function and limits ventricular remodeling after myocardial infarction.

- "Aging Heart and Vessels", Satellite Symposium of the XVII World Congress of the International Society for Heart Research, Melbourne, XJ Du, Ageing and cardiac phenotype development in genetically targeted mice (Invited presentation).

- "Aging Heart and Vessels", Satellite Symposium of the The XVII World Congress of the International Society for Heart Research, H Kiriazis, Effects of aging in mouse heart expressing constitutively active a1B-adrenergic receptors.

- The XVII World Congress of the International Society for Heart Research, Brisbane, XJ Du, Prognostic value and pathologic correlation of echocardiographic indices in aged mice with cardiomyopathy.

- The XVII World Congress of the International Society for Heart Research, Brisbane, XJ Du, Relaxin reverses cardiac fibrosis and inhibits cardiac fibroblast proliferation and differentiation.

- The XVII World Congress of the International Society for Heart Research, Brisbane, XJ Du, Set recruitment and chromatin remodeling are epigenetic components responsible for myocardial gene profiles in the hypertrophied and failing heart.

- The XVII World Congress of the International Society for Heart Research, Brisbane, XJ Du: If channel inhibitor ivabradine lowers heart rate in mice with enhanced sympathoadrenergic activities.

- The XVII World Congress of the International Society for Heart Research, Brisbane, XM Gao, Post-Infarct ventricular rupture is due to matrix collagen damage in mice.

- The XVII World Congress of the International Society for Heart Research, Brisbane, Q Xu, mTld alters subcellular localization of a1A-adrenergic receptors.

- The 8th Congress of Chinese Society of Pathophysiology, China. XJ Du, Role of extracellular matrix in cardiac pathophysiology: recent findings from studies using mice. (Invited lecture)

HUMAN EPIGENETICS LABORATORY

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Research Projects

Fragile X gene (FMR1) transcriptional silencing and the signals to chromatin

A El-Osta, M Chow, H Kn

Fragile X syndrome (FRAXA, OMIM 309550 Online Mendelian Inheritance in Man) is one of the most common forms of inherited mental impairment in humans. There is currently no cure for fragile X individuals. The disease is linked with perhaps the most pertinent of all epigenetic modifications, DNA methylation. This modification to DNA switches the gene off. From an epigenetic standpoint, inactivation of the FMR1 gene is the first of its type to be described by methylation. The mechanisms underlying FMR1 gene regulation and transcriptional repression are unknown and must be investigated if we are to understand the disease. We have identified the molecular determinants involved in FMR1 gene repression and are beginning to fill important gaps in fragile X research. Our data extends the mechanistic link between DNA methylation and fragile X syndrome to provide a new pathway for the inactivation of FMR1. We have established that the transcriptional co-repressors that belong to the methyl-CpG binding protein family play a critical role in FMR1 competence in fragile X disease. One of our goals in the laboratory is to rescue the fragile X phenotype by manipulating transcriptional regulation. We have identified a number of molecular determinants involved in the binding and repression of the fragile X gene. This project also involves somatic knockout of these transcriptional regulators. Our desire to dissect the molecular details of fragile X transcription allows us to determine the role of the chromatin regulators in our attempts to resolve fragile X disease.

Chemotherapeutic drugs have the capacity to remodel chromatin at the mdr1 locus

E Baker, A El-Osta

Chemotherapy induced upregulation of multidrug resistance 1 (MDR1) expression can severely limit the efficacy of cancer treatment. However little is known of the molecular events that control MDR1 activation. We have established that transcriptional activation is dependent on promoter hypomethylation, and chemotherapeutic drug treatment did not result in further demethylation. Dramatic changes in the temporal and spatial patterning of histone modifications were associated with the MDR1 locus in response to drug treatment. Low-level MDR1 upregulation was associated with targeted increases in histone H3 acetylation to a discrete region of the MDR1 locus. Robust increases in MDR1 expression resulted in spreading of H3 hyperacetylation across the MDR1 locus. The H4 acetylation status remained unchanged. Induction of histone H3 K4 methylation was observed only under conditions of robust upregulation of MDR1 expression, or prolonged treatment with Trichostatin A. Our results demonstrate that chemotherapeutic drugs have the capacity to remodel chromatin at the MDR1 locus. A unique interplay of changes in H3 acetylation and H3 K4 methylation indicate that coordinated changes in chromatin architecture are involved in drug-induced activation.

An ATPase dependent chromatin remodeler is a component of the methylation dependent silencing complex

E Baker, S Bassal, H Kn, A El-Osta

DNA methylation is a major determinant in the epigenetic silencing of genes. The mechanisms underlying its targeting and subsequent repression of transcription are relevant to human development and disease. The primary aims of this project are to analyse the functional overlap between the methylation dependent transcriptional repressor, MeCP2 and an ATP-dependent chromatin remodeler. Among the activities that are dependent on methylation and histone deacetylation are the ATPase remodeling complexes that alter chromatin accessibility and transcriptional competence. The mechanisms underlying the correlation between DNA methylation and histone deacetylation have recently been elucidated for MeCP2 (the best characterized member of the methyl-CpG binding domain [MBD] proteins). Evidence suggests that MeCP2/MBD proteins are involved in the recruitment of co-repressor complexes and the assembly of chromatin on methylated DNA. However, the precise mechanism of MeCP2 repression on methylated DNA in the context of chromatin is a hotly debated topic. We show that transcriptional silencing involves the interaction of several components using a variety of different molecular tools. The aim of this project is to delineate the mechanism or modus operandi of MeCP2 mediated repression at the endogenous level. Our data shows that the SWI/SNF binding profile is strikingly similar to that of MeCP2. Knockdown by siRNA suggests that SWI/SNF can reactivate expression

of methylated loci expanding what is currently understood of MeCP2 mediated repression. We propose that MeCP2 is functionally linked with a component of chromatin remodeling. We plan to study this unique partnership between SWI/SNF and MeCP2 transcriptional repression and explore the boundaries of DNA methylation and chromatin remodeling to delineate their functional cooperation. This is paramount in providing the impetus to further our understanding of their roles in human diseases such as tumour suppressor silencing in cancer and mental health disorders such as Rett and Fragile X Syndrome and components of epigenetic memory involved in vascular disease.

The relevance of epigenetic memory in human disease

D Brasacchio, M Cooper, A El-Osta

Until now, almost all activity in the field of gene control focused on the role of transcription factors as the sole participants that regulate gene behaviour. Despite the fact that we have known for almost three decades that DNA is organized into chromosomal domains that control gene expression. These chromosome domains are referred to as "chromatin" and play a central role in all aspects of DNA biology. They are typified by the spectacular molecular events of DNA replication, chromosomal packaging and exquisite transcriptional control. The mechanisms underlying the control of gene expression are dependent on modifications to chromatin and relevant to both human development and disease. Of particular interest to the lab and of therapeutic relevance is understanding how chromatin components regulate NFkB gene expression in diabetic disease. These experimental findings have lead to a re-examination of the mechanistic basis behind NFkB transcriptional repression and brings to prominence the relevance of epigenetic modifications and chromatin perturbations in diabetic complications.

Remodeling of chromatin precedes remodeling of the heart

X-J Du, S Moore, H Kn, A El-Osta

During the development of the heart it is known that a complex repertoire of inductive molecular signals and cardiogenic specific transcription factors are essential for heart specification and determination. Hypertrophied and failing myocardium are characterized of profound changes in gene transcription and molecular remodeling. The mechanisms for targeting and regulation of myocardial genes still remain unclear. Almost all efforts to understand mycardial gene control have focused on the role of transcription factors that control human transcriptional regulatory networks. Our understanding of transcriptional control in recent years have come from scientific advances that represent many disparate disciplines such cancer genetics and epigenetics, remodeling of chromatin during human development and the prominence of epigenetic modifications in disease. These multidisciplinary approaches have emerged from different fields of research and reveal a complex model of gene control. More importantly they forge together a foundation of cell signaling and the transcriptional regulatory circuit and serve as a model for understanding the epigenetic basis of human disease more generally. We now realize that transcriptional response is part of a program of epigenetic control. However, it was not known whether an epigenetic program is involved in myocardial gene regulation during heart failure or the underlying molecular specificity that mediates a transcriptional response. We have experimental evidence that epigenetic information is critical in regulating gene activity in heart disease. The coordinated and ordered response to hypertrophy is remodeling of chromatin and mobilization of the epigenome. We plan to continue research into the functional state of hypertrophy and profile the molecular signature that specifies changes in chromatin dynamics, DNA stability and transcriptional performance.

Grants and other Funding

- Fragile X Research Fellowship (USA), Fragile X gene (FMR1) transcriptional silencing and the signals to chromatin. A El-Osta.

- CFXF Grant (USA), Profiling Chromatin - Dissecting the regulatory binding pattern of Fragile X chromosome in vivo. A El-Osta.

- NMHRC, Characterisation of the anti-apoptotic function of P-glycoprotein and transcriptional regulation of the MDR1 gene. R Johnstone, A El-Osta, J Zalcberg

- NHMRC, A new paradigm for SWI/SNF chromatin function; the ATPase dependent remodeler is a component of the MeCP2 methylation dependent silencing complex. A. El-Osta.

- Middows Foundation, Significance of the epigenetic program in human disease. E Baker, S Bassal, A El-Osta.

Presentations

- 9th International Fragile X Conference, 2004 Washington, DC (Invited)

- Annual Servier Conference, 2004

- University of Illinois at Urbana-Champaign, 2004 (Invited)

- University of California, Department of Biological Chemistry, 2004 (Invited)

- Murdoch Research Institute Melbourne. A El-Osta, Human epigenetics (invited speaker).

- Australian National University Canberra. A El-Osta, Epigenetics in human disease; cancer, mental health and beyond (invited speaker).

- Methylation Matters, 2nd Annual Meeting Sydney Cancer Centre & Transcription Factor Group, NSW. A El-Osta, Methylation and chromatin remodeling in human disease (invited speaker).

MOLECULAR HYPERTENSION LABORATORY

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Research Projects

Aromatase-deficient (ArKO) mice have reduced blood pressure and baroreflex sensitivity

Head GA, Obeyesekere VR, Jones ME, Simpson ER, Krozowski ZS

Aromatase-deficient (ArKO) mice are deficient in estrogens due to deletion of the aromatase gene. We hypothesized that there may be changes in the cardiovascular system of ArKO mice because of evidence linking estrogens with improved cardiovascular outcomes and the induction of the glucocorticoid-metabolizing enzyme, 11beta-hydroxysteroid dehydrogenase type 2 (11betaHSD2), gene in the kidney, which is important for the regulation of blood pressure (BP). BP and baroreflex sensitivity (BRS) in female conscious ArKO mice were compared with those in age- and weight-matched wild-type (WT) mice. Power spectral analysis was used to determine cardiovascular variability and BRS. Although systolic BP was similar in the two groups, diastolic and mean BPs were lower in the ArKO mice (-6.3 +/- 1.9 and -4.6 +/- 2.1 mm Hg, respectively). Heart rate (HR) was greater in the ArKO mice (+36 +/- 6 beats/min). The mean BP in WT mice was 105 mm Hg, and the HR was 481 beats/min. In the autonomic frequency range, BP variability was 74% greater, and HR variability was only 26% that in WT mice. The BRS of ArKO mice was 46% of the value observed in WT mice. 11betaHSD2 levels were unaltered in ArKO mice, except in the kidney, where they were only 10% of WT levels. Estradiol administration to ArKO mice restored renal 11betaHSD2 to WT levels. The results show that ArKO mice have lower diastolic BP, but increased BP variability, perhaps due to an impaired BRS. Thus, aromatase activity is critical for normal autonomic control of the heart and, hence, for reducing the deleterious effects of high BP variability.

Expression of caveolin-1 enhances cholesterol efflux in hepatic cells

Fu Y, Hoang A, Escher G, Parton RG, Krozowski Z, Sviridov D

HepG2 cells were stably transfected with human caveolin-1 (HepG2/cav cells). Transfection resulted in expression of caveolin-1 mRNA, a high abundance of caveolin-1 protein, and the formation of caveolae on the plasma membrane. Cholesterol efflux from HepG2/cav cells was 280 and 45% higher than that from parent HepG2 cells when human plasma and human apoA-I, respectively, were used as acceptors. The difference in efflux was eliminated by treatment of cells with progesterone. There was no difference in cholesterol efflux to cyclodextrin. Cholesterol efflux from plasma membrane vesicles was similar for the two cell types. Transfection led to a 40% increase in the amount of plasma membrane cholesterol in cholesterol-rich domains (caveolae and/or rafts) and a 67% increase in the rate of cholesterol trafficking from intracellular compartments to these domains. Cholesterol biosynthesis in HepG2/cav cells was increased by 2-fold, and cholesterol esterification was reduced by 50% compared with parent HepG2 cells. The proliferation rate of transfected cells was significantly lower than that of non-transfected cells. Transfection did not affect expression of ABCA1 or the abundance of ABCA1 protein, but decreased secretion of apoA-I. We conclude that overexpression of caveolin-1 in hepatic cells stimulates cholesterol efflux by enhancing transfer of cholesterol to cholesterol-rich domains in the plasma membrane.

Demethylation using the epigenetic modifier, 5-azacytidine, increases the efficiency of transient transfection of macrophages

Escher G, Hoang A, Georges S, Tchoua U, El-Osta A, Krozowski Z, Sviridov D

This study was aimed at developing a method for high-efficiency transient transfection of macrophages. Seven methods were evaluated for transient transfection of murine macrophage cells RAW 264.7. The highest transfection efficiency was achieved with DEAE-Dextran, however the proportion of cells

expressing the reporter gene did not exceed 20%. It was subsequently found that the CMV plasmid promoter in these cells becomes methylated. When cells were treated with the methylation inhibitor 5-azacytidine, methylation of the plasmid promoter was abolished and a dose-dependent stimulation of reporter gene expression was observed with expression achieved in more than 80% of cells. Treatment of cells with 5-azacytidine also caused increased efficiency of transfection of macrophages with plasmids driven by RSV, SV40 and EF-1alpha promoters and transient transfection of human hepatoma cells HepG2. Inhibition of methylation also increased the amount and activity of sterol 27-hydroxylase (CYP27A1) detected in RAW 264.7 cells transfected with a CYP27A1 expression plasmid. Treatment of cells with 5-azacytidine alone did not affect either cholesterol efflux from non-transfected cells or expression of ABCA1 and CYP27A1. However, transfection with CYP27A1 lead to a 2-4-fold increase of cholesterol efflux. We conclude that treatment with 5-azacytidine can be used for high-efficiency transient transfection of macrophages.

Contribution of protein kinase A and protein kinase C signalling pathways to the regulation of HSD11B2 expression and proliferation of MCF-7 cells

Rubis B, Grodecka-Gazdecka S, Lecybyl R, Ociepa M, Krozowski Z, Trzeciak WH

Contribution of the protein kinase A (PKA) and protein kinase C (PKC) signalling pathways to the regulation of 11beta-hydroxysteroid dehydrogenase type II (HSD11B2) gene expression was investigated in human breast cancer cell line MCF-7. Treatment of the cells with an adenylyl cyclase activator, forskolin, known to stimulate the PKA pathway, resulted in an increase in HSD11B2 mRNA content. Semi-quantitative RT-PCR revealed attenuation of the effect of forskolin by phorbol ester, tetradecanoyl phorbol acetate (TPA), an activator of the PKC pathway. It was also demonstrated that specific inhibitors significantly reduced the effect of activators of the two pathways. Stimulation of the PKA pathway did not affect, whereas stimulation of the PKC pathway significantly reduced MCF-7 cell proliferation in a time-dependent manner. A cell growth inhibitor, dexamethasone, at high concentrations, caused a 40% decrease in proliferation of MCF-7 cells and this effect was abolished under conditions of increased HSD11B2 expression. It was concluded that in MCF-7 cells, stimulation of the PKA signal transduction pathway results in the induction of HSD11B2 expression and that this effect is markedly reduced by activation of the PKC pathway. Activation of the PKC pathway also resulted in inhibition of cell proliferation, while activation of the PKA pathway abolished the antiproliferative effect of dexamethasone. These effects might be due to oxidation of dexamethasone by the PKA-inducible HSD11B2.

The norepinephrine transporter gene in the postural orthostatic tachycardia syndrome

E Lambert, A El-Osta, L Guo, E Hotchkin, Z Krozowski, A Agrotis, M Schlaich, G Lambert and M Esler

The Postural Tachycardia Syndrome, or POTS (also known as Autonomic Intolerance) has only recently come to international medical attention. The syndrome has multiple symptoms chief among which are symptomatic tachycardia, weakness and recurrent blackouts while standing. The defining characteristic of POTS is an excessive reflex activation of the sympathetic nervous system on standing, with the excessive rise in heart rate and the plasma concentration of the sympathetic nervous system neurotransmitter, norepinephrine, providing the cornerstone of diagnosis. The causes of POTS remain uncertain and are probably multiple. In POTS patients there is consistent evidence for impairment of the process by which each electro-chemical signal in a sympathetic nerve is terminated, the reuptake of norepinephrine back into the sympathetic varicosity by the norepinephrine transporter (NET). In one family kindred the cause of this has been traced to a point mutation in the coding region of the NET gene resulting in the production of a dysfunctional transporter protein. This defect, as expected, was found to augment the sympathoneural signal, most evidently in the heart, and to increase the rate of overflow of norepinephrine into plasma . In the remainder of POTS patients, despite extensive testing, no similar loss of function mutation has been identified and the cause of the phenotype of impaired NET activity remains unknown. We have tested for an alternative cause. Our results lead us to the conclusion that the NET gene promoter region underlies the impairment of NET function phenotype in POTS and is a causal factor in the disorder.

Immunohistochemical distribution of hexose-6-phosphate dehydrogenase and 11b-hydroxysteroid dehydrogenase

C Gomez-Sanchez, A de Rodriguez, D Romero, M Warden, G Fonfara, Z Krozowski and E Gomez-Sanchez

The two 11b-hydroxysteroid dehydrogenase (11-HSD) isoenzymes metabolize the active glucocorticoids cortisol and corticosterone to cortisone and 11-dehydrocorticosterone. The 11-HSD1 has bidirectional activity in homogenates, but has unidirectional reductase activity requiring NADPH as cofactor in most intact cells and tissues. Hexose-6-phosphate dehydrogenase (H6PD) is a microsomal enzyme that provides NADPH for the 11-HSD1. H6PD is known to be co-localized with 11-HSD1 in the liver where 11-HSD1 functions as a oxidoreductase. We found that tissues with highest H6PD mRNA expression relative to 100 % expression in liver were: liver (100), kidney (355), duodenum (151), lung (149), spleen (113), parotid gland (113), adrenal gland (108), subcutaneous fat (107). Tissues with intermediate expression included: muscular stomach (80), seminal vesicle (70), jejunum (62), fallopian tube (47), bladder (45), colon (28), heart (27), thymus (26), brain (23), abdominal aorta (21), cecum (21), mesenteric fat (21), prostate (20) and abdominal fat (18). Tissues with low but detectable levels of H6PD mRNA were: thoracic aorta, skeletal muscle, sartorius muscle, esophagus, diaphragm, trachea, ileum tongue, eyes, skin, and glandular stomach. Immunohistochemical distribution of H6PD was compared to that of the 11HSD1. Liver, kidney, fat, lung, adrenal, colon, pancreas and brain showed somewhat similar distribution of both enzymes. Testis was remarkable in that it expressed very high levels of 11HSD1, but very low levels of H6PD.Western blots showed a single band at a MW of 110kDa in liver and kidney, but more than one band in other tissues. The heart was remarkable in that it showed a single band at 60 kDa. In conclusion, whether 11HSD1 acts as a reductase or dehydrogenase probably depends on the relative expression of the H6PD and the generation of NADPH. This would account for the variations between different tissues of the ratio of the active steroid corticosterone to inactive 11-dehydrocorticosterone.

Modulation of HSD activity in cardiac cells

Z Chai, V Obeyesekere, M Cinel, K Sheppard, S Pepe and Z Krozowski

The HSD1 and HSD2 enzymes play important roles in modulating access of glucocorticoids to the glucocorticoid and mineralocorticoid receptors. Transgenic expression of these enzymes in the heart leads to cardiac fibrosis and heart failure. We have treated cardiac fibroblasts with various cytokines and observed significant modulation of GC metabolizing activity. Furthermore, we have made adenoviral constructs of HSD1, HSD2 and MR and shown biological activity in myocytes and fibroblasts. Cardiac hypertrophy was shown to be modulated by these constructs. These tools will now be used to determine how changes in the levels of these proteins in combination with steroids and cytokines can affect hypertrophy and fibrosis in heart cells in vitro.

Grants and other Funding

- NHMRC, The role of aldosterone in cardiovascular disease. K Sheppard, Z Krozowski.

- Swiss Science Foundation, Cholesterol metabolism in atherosclerosis. G Escher.

Presentations

- West Australian Institute of Medical Research, Z Krozowski, (invited speaker) The role of 11ß-hydroxysteroid dehydrogenases in cardiovascular disease.

MOLECULAR ENDOCRINOLOGY LABORATORY

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Research Projects

EGF receptor transactivation and cardiac hypertrophy

W Thomas, R Hannan, D Onan, N Smith, H-W Chan, L Pipolo, A Jenkins

We continue to examine the molecular mechanisms that underlie Ang II-mediated hypertrophy of cardiac cells. The central paradigm involves an AT1R-mediated activation of metalloproteases that shed EGF receptor ligands from the cell surface of cardiomyocytes, activating EGF receptors and promoting cell growth. We have two main projects: 1) The contribution of Gq coupling of the AT1R to EGF ligand shedding and hypertrophy; and 2) Characterisation of the EGF receptor involved. In the first study, we have used a variety of AT1R mutants, various pharmacological blockers and reporter systems (such as an alkaline phosphatase-tagged HB-EGF reporter construct; HB-EGF-AP) to provide compelling evidence that Gq protein coupling is necessary/sufficient for AT1R mediated HB-EGF shedding and ERK1/2 activation, and may be important in Ang II-induced hypertrophy in cardiomyocytes. In the second study, we have developed adenovirus that express dominant/negative inhibitors of the type 1, 2 and 4 EGF receptors and are using these to selectively inhibit these receptor subtypes in cultured cardiomyocytes to determine their individual contribution to hypertrophy.

Activation and regulation of angiotensin receptors

W Thomas, H Qian, D Dinh, C Oro, L Pipolo

We continue to examine the processes by which arrestin proteins (barrestin 1 and barrestin 2) interact with and regulate the type 1 angiotensin II (AngII) receptor (AT1). We have used receptor signaling assays (inositol phosphate generation, ERK1/2 phosphorylation), receptor binding assays, co-immunoprecipitation, confocal microscopy and proximity assays (such as bioluminescence resonance energy transfer analysis) to examine the molecular mechanisms involved. Most recently, we have been making chimeric versions of barrestin 1 and barrestin 2, where progressively more of the barrestin 1 sequence is substituted into barrestin 2, and using these constructs to identify regions and residues in barrestin 1 and 2 that control selective trafficking to wild type and mutant forms of the AT1 receptor.

Grants and other Funding
- NHMRC, 3 year project grant (2003 –2005), Novel aspects of angiotensin AT1 receptor signalling. M Lew, J Ziogas, W Thomas.

- NHMRC, 5 year project grant (2003-2007), EGF receptor trans-activation in GPCR-mediated cardiac hypertrophy. W Thomas, R Hannan.

- NHMRC, 3 year project grant (2004-2006), Arresting angiotensin receptors. W Thomas, I Smith.

- NHMRC, 3 year project grant (2004-2006), The physiological and molecular regulation of ECE subcellular distribution and vascular endothelin production, I Smith, R Lew, W Thomas, P Little.

- NHMRC, 3 year project grant (2005-2007), Helix VIII of G protein coupled receptors as a lipid-activated signalling sensor, M Aguilar, W Thomas.

Presentations
- Annual Meeting of the Australian Society for Biochemistry and Molecular Biology (Perth, WA) W Thomas, EGF receptor transactivation by 7TM receptors in cardiac hypertrophy.

- St Vincent's Institute of Medical Research (Vic) W Thomas, EGF receptor transactivation and cardiac hypertrophy.

- Molecular and Cellular Targets in Tissue Remodelling Symposium (Melb Vic) W Thomas EGF receptor transactivation and cardiac hypertrophy.

- Host and Session Chair of Peptide Users Group (Victoria) autumn meeting AMREP seminar room, March 30, 2004. W Thomas.

- Ludwig Institute for Cancer Research, June 4, 2004. W Thomas, EGF receptor transactivation and cardiac hypertrophy.

- School of Biomedical Sciences Seminar Series. University of Queensland, March 19, 2004. W Thomas, Arresting Angiotensin Receptors.

- Angiotensin Gordon Research Conference. Ventura Beach, California, February 29 - March 5, 2004. W Thomas, invited Session Chair Structure-function of AT1 receptors.

- Angiotensin Gordon Research Conference, Ventura Beach, California, February 29-March 5, 2004. W Thomas, invited Signalling Session.

- Angiotensin Gordon Research Conference, Ventura Beach, California, February 29-March 5, 2004 H Qian, N Smith, invited presentations.

CELLULAR BIOCHEMISTRY LABORATORY

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Research Projects

Phospholipase Cb in cardiomyocyte growth

O Vasilevski, E Woodcock

The receptor coupling protein Gq is necessary for the development of myocardial hypertrophy under conditions of pressure or volume overload in vivo. We have made a detailed study of signalling responses initiated by Gq in cardiomyocyte model systems. Activation of Gq by the a1-adrenergic receptor agonist phenylephrine is well known to cause cell growth as well as 'hypertrophic' marker gene expression. In the studies performed in 2004, we investigated whether phospholipase Cb isoforms were critical for these Gq- mediated responses. To do this we developed a novel adenoviral vector that can express inhibitory RNA species that selectively reduce the two isoforms of phospholipase Cb, b1 and b3. We have now generated these inhibitory viruses ready for experimentation in isolated cardiac cells and in rat and mouse heart in vivo.

Sarcolemmal changes Ins(1,4,5)P3 generation and arrhythmogenesis

L Turnbull, X-J Du, E Woodcock

We have previously shown that myocardial ischaemia as occurs during a 'heart attack' is associated with generation of the calcium-releasing messenger Ins(1,4,5)P3. We also showed that Ins(1,4,5)P3 was essential for the initiation of arrhythmias and sudden death under these conditions. In studies performed in 2004 we showed that ischaemia causes changes to regions of the plasma membrane of the cardiomyocytes that are involved in receptor signalling. The precursor of Ins(1,4,5)P3, PIP2, was increased in this membrane fraction during ischaemia. In addition the enzyme responsible for Ins(1,4,5)P3 generation, phospholipase cb1, was retained in these signalling complexes, where it can increase Ins(1,4,5)P3 above levels generated under physiological conditions. We also showed that the receptors for noradrenaline that are central to this process are actually localized outside the signalling complexes. This likely explains their low activity under physiological conditions. These changes in the functioning of signalling complexes in the plasma membrane most likely are responsible for the enhanced Ins(1,4,5)P3 generation and arrhythmogenesis observed under these conditions.

Regulation of FOXO activation in cardiomyocytes

H Huynh, B Kenney, E Woodcock

FOXO proteins are transcription factors involved in regulation of cell cycle, glucose utilization and apoptosis. They are regulated by phosphorylation by such factors as insulin and epidermal growth factor (EGF) via a protein kinase called Akt. Akt is an important factor in cardioprotection following ichaemic insult and so understanding how Akt provides protection is of importance. We have shown, for the first time, that FOXO proteins are expressed and regulated in cardiomyocytes and that they are active in inducing pro-apoptotic gene transcription. FOXO1 was phosphorylated by Akt, EGF and insulin, and dephosphorylated by activation of Gq-coupled receptors. Aky also reduced expression of FOXO1, while surprisingly increasing expression of FOXO4. Dephosphorylation of FOXO1 caused translocation into the nucleus and subsequent activation of genes encoding pro-apoptotic effectors such as tumor necrosis factor a and Fas-ligand. This shows that in addition to inhibition of the mitochondrial pathway of cardiomyocyte apoptosis, Akt activation protects by reducing the synthesis of pro-apoptotic cytokines.

Grants and other Funding

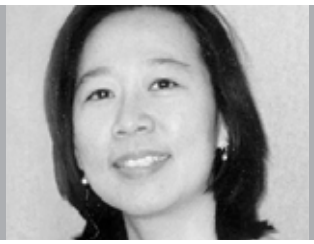
- NHMRC, Inositol polyphosphate 1-phosphatase, a novel anti-hypertrophic factor. E Woodcock, I Smith.

- NHMRC Ischaemia-induced sarcolemmal changes and their role in Ins(1,4,5)P3 generation and arrhythmogenesis. E Woodcock, L Turnbull.

- NHMRC FOXO proteins and protection from cardiac ischaemic injury. E Woodcock, R Lin, I Dawes, A El-Osta.

- Rebecca Cooper Foundation. The use of RNA-interference technology to define targets for anti-arrhythmic therapy. E Woodcock.

VASCULAR PHARMACOLOGY LABORATORY



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Research Projects

Shear-dependent Role for Soluble P-selectin in Regulating Neutrophil Adhesion to Platelets

Woollard KJ1, Dart A1, Jackson S2, Kling D3 and Chin-Dusting J1
1. Wynn Domain, Baker Heart Research Institute, Melbourne, Australia
2. Australian Centre for Blood Disease, Monash University, Melbourne, Australia
3. F-Hoffman La Roche, Basel, Switzerland

Introduction: P-selectin supports adhesion of leukocytes to activated platelets present in thrombi or to activated endothelial cells. A soluble form of P-Selectin is found at elevated levels in the plasma of patients with vascular disorders. It is potentially active because it possesses the lectin and epidermal growth factor domains required to bind its receptor, PSGL-1, expressed by leukocytes. However its functional significance is not yet clear.Methods: Neutrophils were incubated with soluble P-Selectin with or without anti-PSGL-1 antibody, before application to spread platelet monolayers or a fibrinogen matrix. In some experiments neutrophils were analysed for CD11b activation via FACS. Neutrophil adhesion with or without shear flow was recorded and analysed using standard phase contrast microscopy. Results: Soluble P-Selectin dose-dependently (75-150ng/ml) increased neutrophil adhesion to fibrinogen and platelet mo nolayers, however adhesion to a fibrinogen matrix was significantly higher (212.5%±31.4; n=4) than on spread platelet monolayers (123.5%±27.6; n=4; P<0.01). This increase in adhesion was due to a higher proportion of tethered neutrophils forming stable adhesive contacts with the platelet surface, which correlates with soluble P-selectin mediated Mac-1 activation on the surface of neutrophils. This increase in adhesion was dependent on both PSGL-1 binding and Src kinase activation. Soluble P-selectin mediated neutrophil adhesion to platelet monolayers was inversely correlated with shear. At low shear (50s-1) there was a 92.7%±15.7 (n=4) increase in adhesion, which reduced to 38.5%±11.9 (n=4) at 150s-1 and 10.1%±8.9 (n=4) increase at 300s-1. These results suggest a potentially important role for soluble P-selectin in promoting neutrophil – platelet adhesive interactions, particularly under low shear conditions. Conclusion: These studies define a novel role for soluble P-selectin in modulating neutrophil adhesion to platelet monolayers in a shear regulated manner.

MD-960 induces Vasodilatation in Rat Isolated Aortic Rings via a Nitric Oxide Dependent Pathway

M. Vincent1, P. Jenkins2, J. P.F. Chin-Dusting1
1. Vascular Pharmacology, Baker Medical Research Institute, Prahan, Australia
2. Monash University, AusBio Ltd, 3800, Clayton, VIC, Australia

MD-960 is novel therapeutic compound developed by AusBio Ltd for the treatment of diabetes mellitus, insulin resistance and the metabolic syndrome. To characterise the vascular profile of MD-960, full concentration-response curves (1 _M-1000 _M) to the compound were obtained on rat isolated aortic rings pre-constricted with submaximal concentrations of noradrenaline. This was done in endothelium intact and denuded vessels and also in the absence and presence of any one of the following inhibitors (n>6 in any one group): indomethacin (3 _M, a cyclo-oxygenase inhibitor),(-)-perillic acid (30 _M, a p21(ras) blocker), 2-[2'-amino-3'-methoxy-phenyl]-oxana-phthalen-4-one (PD 98059) (10 _M, a p42/p44 mitogen-activated protein kinase inhibitor), 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole (SB 203580) (1 _M, a p38 mitogen-activated protein kinase blocker), wortmannin (500 _M, a phosphatidylinositol-3 kinase inhibitor), genistein (10 _M, a tyrosine kinase blocker), 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) (10 _M, an inhibitor of soluble guanylyl cyclase) and NG-nitro-L-arginine (10 _M; an inhibitor of nitric oxide synthase). No two interventions were used on the same aortic ring from any one rat. The neg log EC50 value for MD-960 in intact vessels (mean + sem: -4.38 + 0.22 was significantly shifted to the right by endothelial denudation (-2.35 + 0.35; p<0.05), NG-nitro-L-arginine (-2.35 + 0.35) and ODQ (-3.95 + 0.33). Similarly, a statistically significant decrease in the maximum response to MD-960 (72.71 + 9.86% vasodilatation) was observed in the presence of NOLA (37.32 +/-7.46; p<0.05) and ODQ (14.83 +/-5.68; p<0.05). No significant change in either neg log EC50 values or the maximum % relaxation for MD-960 was observed in the presence of any of the other inhibitors examined. Conclusion: MD-960 exerts a vasodilatory action via inducing endothelial release of nitric oxide which subsequently activates soluble guanylate cyclase on smooth muscle cells. The demonstrated

vasodilatory effect of MD960 may be of therapeutic benefit in patients with metabolic syndrome.

nor-NOHA IS A NITRIC OXIDE SYNTHASE INHIBITOR IN RAT MESENTERIC ARTERIES

N-N Huynh1*, EE Jones*, J Chin-Dusting*.
Vascular Pharmacology, Wynn Domain, Baker Heart Research Institute*, Melbourne and Department of Medicine, Central and Eastern Clinical School, Monash University, Alfred Hospital1.

Background: Nw-Hydroxy-L-arginine (L - NOHA), is an endogenous intermediary for nitric oxide production and reported to be an inhibitor of arginase. Nw-Hydroxy-nor-L-arginine (nor - NOHA), is a synthetic analogue of L-NOHA which reportedly selectively inhibits arginase but does not act as a substrate for NO. 1. We examined the functional selectivity of these compounds in rat isolated mesenteric arteries.
Methods: Mesenteric arteries from male Sprague-Dawley rats were excised and mounted in Mulvany myograph chambers and normalised to transmural pressure of 90 mmHg. Full concentration response curves to L- NOHA and nor-NOHA (± endothelium, ± 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, a cyclic GMP inhibitor at 10mM in denuded vessels only)), ACh ± L-NOHA (10 mM) and nor-NOHA (200 mM), ACh ± nor-NOHA (200 mM) ± L-arginine (100 mM) and SNP ± L- NOHA (10 mM) and nor-NOHA (200 mM) were obtained on 40mM KCl pre-constricted vessels. Results are presented as % relaxation to 40mM KCl.
Results: Responses to both L-NOHA (max dilatation: denuded vs intact; 61.2 ± 3.28% n = 7 vs 48.5 ± 4.33%; n = 4; $p<0.05$) and nor-NOHA (log EC-50: denuded vs intact; -3.68 ± 0.16 vs EC50 = -4.51 ± 0.27; n=7 each; $p<0.05$) were significantly enhanced in endothelial denuded vessels and significantly diminished with addition of ODQ ($p < 0.01$). Nor-NOHA, but not L-NOHA, significantly depressed responses to acetylcholine (n=8; pre vs post nor-NOHA: max dilatation: 66.51 ± 4.734 %, log EC50 = -7.65 ± 0.31 vs 30.80 ± 3.70%, log EC50 = -7.26 ± .258). This effect of nor-NOHA was reversed with addition of L-arginine. Responses to SNP were not affected by either L-NOHA or nor-NOHA.
Conclusion: L – NOHA and nor-NOHA cause vasodilatation through direct stimulation on vascular smooth muscle via a cGMP dependent pathway. Nor-NOHA, but not L-NOHA, also inhibit responses to acetylcholine, an inhibition which can be overcome by addition of L-arginine suggesting that nor-NOHA is an eNOS inhibitor. These findings have not previously been reported of either compounds and severely limit their use as selective arginase inhibitors.

1. Boucher JL, et al. Cell Mol Life Sci. 1999; 55 (8-9): 1015-28.

Neither Responses to Exogenous nor Endogenous Endothelin-1 are Altered in Patients with Hypercholesterolemia

Chin-Dusting J, Boak L, Duffy S and Dart A.
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Although evidence for an activated endothelin system is well documented in several cardiovascular disease states and in some patient groups at high risk of cardiovascular disease, this is less clear for patients with high plasma cholesterol levels. Since both opposing studies (Cardillo et al, 2000; Nohria et al., 2003) used endothelin antagonists at doses documented to be non-selective (Goddard and Webb, 2002) therefore confounding data interpretation, the current study aimed to re-dress this limitation. 22 patients with high plasma cholesterol levels (Hc; total cholesterol: 7.33 + 0.39 mmol/L); and 17 age and sex-matched controls (C; total cholesterol 4.91 + 0.16 mmol/L) were recruited. All underwent at least one or more of the following drug infusion (via brachial artery) protocols, each performed on a separate day: 60 min infusion endothelin-1 5 pmol/min; 60 min ETA antagonist BQ-123 10nmol/min; 60 min ETB antagonist 1nmol/min. Forearm vascular responses to these drugs were obtained using venous occlusion plethysmography. Plasma endothelin-1 levels (by ELISA) were not significantly different in the two groups (Hc vs C: 1.06 + 0.15 vs 1.16 + 0.18 fmol; p=0.64). Endothelin-1 caused a vasoconstrictory response in both groups ($p<0.001$) but this was not significantly different between groups (p=0.899). BQ-123 caused a vasodilatory response in both groups ($p<0.01$) but this was not significantly different between groups (p=0.899). BQ-788 did not induce a clear effect on forearm blood flow in either group; there was no statistical difference in the response to this drug between the two groups (p=0.744). We conclude that neither responses to endogenous nor exogenous endothelin are altered in patients with hypercholesterolemia.
Cardillo et al. J Am Coll Cardiol 2000;36:1483-8. Nohria et al. Hypertension 2003;42:43-48. Goddard and Webb. Hypertension 2002;40:e1.

The role of advanced glycation endproducts in mediating vascular responses to acetylcholine and endothelin in the rat aorta

Jones EE, Chin-Dusting JPF, Arnstein M, Cooper ME, Forbes JM.
Baker Heart Research Institute, Wynn Domain, Melbourne.

Background: The accumulation of advanced glycation endproducts (AGEs) is believed to contribute to the chronic complications of diabetes mellitus.1 We aimed to investigate the effect of elevated AGEs on vascular responses within rat aorta and further to explore whether these effects could be influenced by administration of the AGE cross-link breaker, ALT-711 or the mitochondrial ROS scavenger, idebenone.
Methods: 8 week old male Sprague Dawley rats (n=10/group) were administered either AGE-RSA (rat serum

albumin) or RSA (20mg/kg/day), or saline (SHAM) for 16 weeks. Two additional subgroups received ALT-711 (10mg/kg/day oral gavage) or idebenone (100mg/kg/day i.p.) as well as the AGE-RSA or RSA treatments. For comparative purposes diabetes was induced in a separate group of rats using steptozotocin (50mg/kg i.v.) for 16 weeks. At the end of 16 weeks rats were culled by exsanguination and thoracic aortae removed, dissected into 2 mm segments and mounted in standard organ baths. Full cumulative concentration-response curves to endothelin (ET-1), sodium nitroprusside (SNP) and acetylcholine (Ach) were constructed.
Results: Responses to Ach were dampened in aortic rings from streptozotocin rats, however did not reach significance (p=0.1). Although responses to Ach trended towards an augmentation with AGE-RSA (Emax=94±5% vs Emax=77±7% (p=0.1); AGE-RSA vs control respectively) and appeared to normalise with ALT-711 (Emax=85±4%)) these findings were not statistically significant. Neither responses to sodium nitroprusside nor endothelin-1 were altered in any of the treatment groups.
Conclusion: That 16 weeks of AGE-RSA treatment does not mimic the vascular profile observed with diabetes induced by streptozotocin for 16 weeks, suggesting that effects on dilatation may be glucose mediated.
1. Makita Z, et al. N Engl J Med. 1991;325(12):836

Grants and other Funding

- NHMRC Program Grant, Heart failure and its antecedents. G Jennings, M Esler, A Dart, D Kaye, A Bobik, J Chin-Dusting, B Kingwell, X-J Du.

- Clinical Cardiovascular Centre of Excellence. G Jennings, M Esler, A Dart, D Kaye, J Chin-Dusting, B Kingwell, C Reid.

- Singapore NMRC, Identifying the L-arginine Transporter and Targeting Patients as High Cardiovascular Risk with eNOS Polymorphisms for Therapeutic Restoration of Endothelium Function: Implications of Ethnicity. J Chin-Dusting, K Tian Hok.

- CASS Foundation, Effect of Probiotics on Cardiovascular Complications in Human Cirrhosis. J Chin-Dusting, N Connelly.

Commercially Funded Research Activities

- Hoffmann-La Roche, Soluble P-selectin in peripheral occlusive arterial disease - an educational grant. J Chin-Dusting, D Kling (Roche).

- Actelion Ltd, Role of nitric oxide in thrombus formation – an educational grant. J Chin-Dusting

- Aus-Bio Ltd, Vasodilatory profile of MD-960. J Chin-Dusting

Presentations

- Probiotics and the Digestive Tract, Rome, Italy. Chin-Dusting J (invited) Expert Delegate.

- Queensland University of Technology,Biological Sciences, Brisbane, May. Woollard K. Soluble P-selectin and vascular disease.

- Royal Melbourne Institute of Technology, School of Health Sciences, Melbourne, Sept. Chin-Dusting J Circulatory Complications in Cirrhosis.
- European Society of Hypertension, France, Paris, June. Chee R, Kaye D, Chin-Dusting JPF, Moe K
PPAR gamma agonists and the role of VEGF in the development of oedema.

- European Society of Hypertension, France, Paris, June.
Australian Society of Thrombosis and Haemostasis, Melbourne
Soluble P-selectin increases neutrophil adhesion to spread platelet monolayers
Woollard K, Jackson S, Kulkarni S, Kling, D and Chin-Dusting J.

- Australian Health and Medical Research Congress, Darling Harbour, Sydney. Ngan, Huynh and Chin-Dusting J nor-NOHA inhibits Acetylcholine responses in rat mesenteric arteries.

- Australian Health and Medical Research Congress, Darling Harbour, Sydney. M. Vincent, P. Jenkins, J. P.F. Chin-Dusting. MD-960 induces Vasodilatation in Rat Isolated Aortic Rings via a Nitric Oxide Dependent Pathway.

- Australian Health and Medical Research Congress, Darling Harbour, Sydney. The role of advanced glycation end products in mediating vascular responses to acetylcholine and endothelin in the rat aorta
Jones EE, Chin-Dusting JPF, Arnstein M, Cooper ME, Forbes JM.

- Australian Health and Medical Research Congress, Darling Harbour, Sydney. Elevated HDL Cholesterol is Functionally Ineffective in Cardiac Transplant Recipients: Evidence for Impaired Reverse Cholesterol Transport
D. Sviridov, J. P.F. Chin-Dusting, P. Nestel, B. Olchawa, A. Hoang, J. Starr, A. Dart.

- Australian Health and Medical Research Congress, Darling Harbour, Sydney. Evaluation of FcgammaRIIa-H/R polymorphism by allele-specific primer polymerase chain reaction and its functional response to C-reactive protein
Woollard K.

CARDIAC SURGICAL RESEARCH LABORATORY

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Olivier Van den Brink - PhD (Monash)

Research Projects

Defining Enkephalin Opioid Peptide Metabolism in the Human Heart

O van den Brink, R Denver, D Quick, A.Calderone, L Delbridge, F Rosenfeldt, S.Marasco, M.Rowland, D Esmore, D Kaye, and S.Pepe

The role of enkephalin opioids in the healthy or diseased human heart is not well defined. We have found that enkephalins, when delivered exogenously, preserve developed force capacity in isolated human myocardial trabeculae after hypoxic injury in vitro, by reducing myocardial oxygen-energy demand. This project involves defining the expression of proenkephalin gene mRNA, related post-translational protein products, and abundance and localisation of opioid peptide receptor subtypes in the human heart and vasculature obtained from biopsies, brain dead donors and non-cardiovascular disease-related post mortem samples. These measures are contrasted to data from explanted failing hearts. Assay development and validation is complete. Sample assays are almost complete. Immunohistochemical analyses of heart sections are close to completion.

Sub-studies Preliminary data:
1) We have measured enkephalin opioid peptides released into the coronary sinus from the heart in catheter laboratory heart failure patients. These patients are also subjected to tilt-table stress to evoke a sympathetic nervous system response. There is a marked reduction in myocardial and coronary sinus methionine-enkephalin in end-stage heart failure that is associated with increased noradrenaline "spill-over".

2) Patients undergoing elective "on cardiopulmonary bypass" CABG surgery exhibit significantly depressed plasma methionine-enkephalin levels. Our study demonstrates that cardiac ischemia during cardioplegia and reperfusion in heart surgery acutely increases coronary sinus and arterial plasma content of methionine-enkephalin, however these plasma levels remain lower than in samples from healthy subjects. These data suggest a pathophysiological role and potential therapeutical window for the regulation of methionine-enkephalin.

3) In order to separate neural from myocardial release enkephalin release, we examined met-enkephalin release from donor hearts that have been transplanted into recipients. Measurement of met-enkephalin (concurrent with noraderenaline) is made prior to the expected reestablishment of the neural connections in the heart that were severed during explantation. Comparison with coronary sinus measures of enkephalin taken after reinnervation of the heart will shed light on the extent of neural afferent versus cardiac contribution of enkephalin.

4) We have also explored enkephalin expression during the progression of hypertrophy in a transgenic mouse model (AOGN) of cardiac hypertropy (overexpression of cardiac angiotensinogen-see below). We have demonstrated augmented myocardial enkephalin expression with increased age and growth of wild type mice. In contrast, the AOGN mice have depressed enkephalin expression that increases modestly and dis-proportionally to the rate of growth and hypertrophy. To distinguish between hormonally versus metabolically induced cardiac hypertrophy we also studied another transgenic mouse model that has the gene for the Glucose transporter4 knocked out (Glut-4-KO). Interestingly, despite severe cardiac hypertrophy in this model we could not establish a link with the enkephalin system.

Altered Protein Expression of Mitochondrial Respiratory Complexes in Human Heart Failure

F.Sheeran, D.Quick, A.Calderone, F.Rosenfeldt, S.Pepe.

We have developed methodology to examine, in human donor hearts or explanted end-stage failing hearts, the expression of key mitochondrial proteins responsible for oxidative

phosphorylation, using monoclonal antibody specific recognition in SDS PAGE. The aims of the project are to detect altered expression of mitochondrial proteins during heart failure and to determine whether potential therapeutic drugs, alter their expression and functional capacity. In addition to quantitative expression of isolated mitochondrial complexes and their subunits, we aim to determine which of these are functionally limited due to formation of cross-link adducts after reacting with peroxidative products such as 4-hydroxynonenal.
Mitochondrial and nuclear mRNA expression for the proteins that are adversely affected by formation of these adducts will be measured. We aim to determine whether clearance and turnover rates of these products can be manipulated to improve mitochondrial function in failing hearts. Mitochondrial complexes I and III are most affected by mitochondrial superoxide and peroxidative products. It may be possible to design new therapies to augment mitochondrial biogenesis generally or to increase expression of key functional complexes and intramitochondrial enzymes that remove inactivated or dysfunctional proteins using specific adenovirus transfection strategies.

Functional Rescue of Hypertrophic Transgenic Mice With Chronic Overexpression of Cardiac Angiotensinogen Via Modulation of Cardiac Membrane Omega-3 Polyunsaturated Fatty Acids

C.Huggins, L.Delbridge, P.McLennan, S.Pepe.

Our previous work with transgenic mice harbouring multiple copies of the cardiac-specific angiotensinogen transgene demonstrate that chronic overproduction of cardiac AngII, independent of changes in blood pressure, may induce hypertrophic ventricular remodelling that precedes impaired cardiac function and failure. We have also shown earlier that augmenting omega-3 to omega-6 polyunsaturated fatty acid (PUFA) ratio in cardiac membrane phospholipids leads to: reduced vulnerability to proarrhythmic stimuli; reduced incidence of arrhythmogenesis; greater capacity to recover contractile function after ischemia-reperfusion injury and; improved mitochondrial oxygen utilisation efficiency. Our aim is to examine whether remodelling of the cardiac membrane PUFA are modified in this transgenic model of hypertrophy (AOGN mouse), and whether cardiac membrane remodelling to increase omega-3 PUFA content may ameliorate the dysfunctional contractile performance and abnormal expression of contractile proteins evident in these transgenic mouse hearts.
We have found that cardiac membrane phospholipid fatty acid composition of omega-3 fatty acids are augmented from 21.9±1.3% to 46.5±0.6% (of total fatty acids) in AOGN after four weeks of dietary treatment. Preliminary data from isolated heart perfusion experiments demonstrate that in young AOGN, when the hypertrophic phenotype is not maximally developed, cardiac performance is adapted to coping with isolated heart perfusion stress. It is only when the hypertrophic phenotype has fully emerged that elevation of cardiac membrane omega-3 PUFA leads to improved contractile function of isolated perfused AOGN hearts, particularly after ischemia and reperfusion. These experiments are still in progress.

Inhibition of P38 MAPK Inhibition on donor brain death and donor heart preservation for transplantation

T Oto, A.Calderone, F Rosenfeldt, S Pepe

Brain death is unavoidable in the donor heart transplantation process. Brain death triggers a marked release of catecholamines causing coronary vasoconstriction and ischemia and also evokes rapid expression of hormones and inflammatory mediators that adversely affect long term survival of the transplanted heart. We have established a model of rapid onset brain death to better assess donor heart function in studies that test the cardiac effects of anti-inflammatory agents on the molecular consequences of brain death and during donor heart storage and implantation. We are examining the use of P38 mitogen activated kinase inhibitors to limit cytokine-induced cardiac injury to donor hearts preserved for transplantation.

Prospective Randomised clinical Trial of a Preoperative combination of Metabolic Physical and Mental Therapy (MPM) for high risk cardiac surgery patients

J-Y Leong, S Pepe, J Van der Merwe, D Esmore, FL Rosenfeldt

This study is aimed at evaluating a preoperative metabolic, physical and mental program in patients undergoing cardiac surgery at the Alfred Hospital. For two or more weeks preceding cardiac surgery patients receive a daily preparation of antioxidants (alpha-lipoic acid, coenzyme Q10 and selenium), magnesium orotate and omega fatty acids, and receive exercise training and mental stress reduction therapy. Quality of life assessments and blood measures of lipid peroxidation are made before treatment, immediately before surgery and one month after surgery. A pilot study carried out in 2003 in 16 patients showed benefits of this therapy compared to historical controls. MPM treated patients showed reduced oxidative stress, improved quality of life one month after surgery and a hospital stay shortened by one day. The current prospective randomised clinical trial will run at The Alfred for another 18 months and it is planned that patients will also be recruited from the Monash Medical Centre and the Austin Hospital.

Skeletonised Mammary Artery Reduces Tissue Trauma (SMARTT) trial

J-Y Leong, P Markman, M Rowland, FL Rosenfeldt

This prospective randomised study (started in 2002) investigates the possibility of reducing the long-term anterior chest wall pain and sensory defect that may accompany sternotomy and harvesting of the internal mammary artery. A new technique (Skeletonised Internal Mammary Artery) has been used which minimises trauma to the chest wall including the intercostal nerve. Patients are randomised to receive either the standard harvesting technique or the new technique. Pain and neural function in the chest is compared between the two groups. Preliminary results have shown that diabetic patients have greater sensory loss on their chest wall than non-diabetics. An unexpected finding was sensory loss on the side of the chest without the harvesting procedure. Measures of sensory loss

have now been expanded to detect damage produced by spreading the ribs that may cause traction injury to the intercostal nerves adjacent to the vertebral column.

Trial of Implantation of the EPIC Tissue Heart Valve

M Rowland, FL Rosenfeldt, M Nicholls, S Marasco, A Pick, J Negri, D Esmore

This new heart valve has recently been introduced to Australia by St Jude Medical who are sponsoring a multi-institution, national trial. The Alfred has implanted 30 patients with good clinical success. Follow up of patients at one year shows excellent results.

Prospective Randomised Clinical Trial of Coenzyme Q10 for Heart Failure – Q Symbio Trial

FL Rosenfeldt, M Nicholls, J Van der Merwe, D Kaye, P Bergin, M Richardson

This multinational clinical trial concerns patients with severe heart failure who are treated with Coenzyme Q10. F Rosenfeldt is one of the principal investigators. So far six patients have been enrolled at The Alfred. The trial will continue through 2004-2005.

Preservation of Donor Lungs by Airway Cooling

T Oto, S Pepe, FL Rosenfeldt

A new group of donors for lung transplantation has been identified. These are patients having a cardiac arrest in the hospital environment. The lungs can be obtained from these patients and transplanted prior to damage occurring to the lung tissue. We have developed a method of protecting the lungs from damage while still in the body by insufflating the lungs with very cold air. Preliminary data has shown good preservation of lung function and normalised metabolism within the lung compared to untreated controls.

VentrAssist Artificial Heart

D Esmore, D Kaye, FL Rosenfeldt, R Salamonsen

Ten patients have now been implanted at The Alfred Hospital. The first implanted patient survived for 18 months then died from complications unrelated to the pump. Three patients are currently implanted and are surviving up to 12 months. Six have died or been transplanted. The pump has now entered the phase of national and international trials with many new iimplantation sites around Australia, in Europe and in the United States.

Grants and other Funding

- National Institutes of Health, National Institute on Aging, USA. R13 Grant. Aging Heart & Vessels. J Downey, S Pepe.

- NHMRC, Enkephalin metabolism in cardiac ischemia, heart failure and cardiac surgery. S Pepe, L Delbridge, F Rosenfeldt.

- NHMRC, Enhancing the cardioprotective effect of diadenosine tetraphosphate: designing inhibitors of Ap4A hydrolase. P Gooley, S Pepe, K Gayler, D Swarbrick.

- NHF, Omega-3 dietary lipids in genetic hypertrophy. L Delbridge, S Pepe, P McLennan.

- Australian Research Council, Tissue valve engineering. Y Morsi, F Rosenfeldt.

- Australian Research Council, Engineering Blood Vessels. Y Morsi, F Rosenfeldt.

- Blackmores Pty Ltd, Metabolic supplementation in surgery. F Rosenfeldt.

- St Jude Medical Pty Ltd. F Rosenfeldt, D Esmore.

Commercially Funded Research Activities

- St Jude Medical, EPIC heart valve trial. D Esmore, et al. Q Symbio Trial. Pharmanord, Denmark. Rosenfeldt, et al.

Presentations

- XVII World Congress of the International Society for Heart Research, Brisbane. Invited Speaker & Co-chair, August 9, 2004. Omega-3 Fatty Acids-Optimising Cardiac Function in Health & Disease: Targeting mitochondrial function & cardiac metabolism. S. Pepe.

- Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, USA, Invited seminar presentation. November 17, 2004. Opioid peptides in the human heart. S. Pepe.

- National Ageing Research Institute, Melbourne University. Invited seminar presentation. Melbourne, May 17, 2004. Age-related modification of mitochondrial membranes & respiratory function. S. Pepe.

- National Ageing Research Institute, Melbourne University. Invited seminar presentation. Melbourne, May 17, 2004. Metabolic Therapy for the Aging Heart. F Rosenfeldt.

- Department of Biochemistry, La Trobe University. Invited seminar presentation. Melbourne, April 27, 2004. Opioid peptide expression and function in the human heart. S. Pepe.

- XVII World Congress of the International Society for Heart Research, Brisbane. Invited Speaker & Co-chair. August, 2004. Myocardial Preservation. F Rosenfeldt.

- Symposium on Aging. University of Halle. Invited seminar presentation. Ageing Heart & Cardiac Surgery. F Rosenfeldt.

- Aging Heart & Vessels Satellite Conference. Melbourne. Invited Speaker & Chair, Metabolic Therapy for Heart Disease. F Rosenfeldt.

- International Society for Heart & Lung Transplantation, San Francisco, USA. Lung transplantation methodologies. T Oto.

APPLIED CARDIOVASCULAR RESEARCH LABORATORY

John Power

Head
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Dr Masahiko Hoshijima - PhD, UCSD, San Diego, CA, USA

Students
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Paul Gould - PhD (Monash)

Research Projects

Percutaneous Cardiac Gene Delivery

J Power, M Byrne, A Bilney, P Horton, F Fitzpatrick, DM Kaye

We have developed a percutaneous system for the exclusive delivery of vectors containing genetic material to the heart in both normal and heart failure animals. This system has been used in collaborative studies with Prof Ken Chien and colleagues from the UCSD. The system and methods are the subject of two patent applications. A start up company V-Kardia Pty Ltd has been formed to exploit this technology.

Percutaneous Mitral Annuloplasty Device

J Power, M Byrne, P Horton, F Fitzpatrick, DM Kaye

The pre clinical portion of this project has been completed. A second paper with chronic implant data was published in Circulation. The first human acute implants were done at the Alfred Hospital. The first series of chronic implants will start in Germany in Feb 2005.

A role for cardiac pacing in limiting post ventricular myocardial infarct expansion

J Power, M Byrne, P Horton, F Fitzpatrick, D Kaye

In this study we have examined a new role for cardiac pacing following myocardial infarct. We were able to show that pacing restricts the degree of post infarct remodeling. Publications are expected early in 2005.

Percutaneous Tricuspid Annuloplasty Device

J Power, A Bilney, P Horton, F Fitzpatrick, DM Kaye

We have experimented with a new percutaneous approach to the treatment of tricuspid valve regurgitation. One patent has been applied for from this work and animal studies are continuing.

Grants and other Funding
- NHMRC, Program Grant "Heart Failure and its antecedents".

Commercially Funded Research Activities
- Sunshine Heart Company, Peri-Aortic Counterpulsation. P Horton, M Byrne, F Fitzpatrich, J Power.

- Cardiac Dimensions Inc, Percutaneous Mitral Annuloplasty. D Kaye, M Byrne, P Horton, J Power.

- Guidant Corporation, Pacing and Myocardial Infarction. M Byrne, P Horton, F Fitzpatrick, J Power.

Presentations
- Annual Scientific Sessions of the European Society of Cardiology. Munich JPower, Chronic effects of percutaneous annuloplasty in heart failure.

BRAIN - CARDIOVASCULAR NEUROSCIENCE DIVISION

Murray Esler

The brain-heart link is the primary focus of research for this Division, investigated both in humans and in experimental animals. The Division incorporates the Human Neurotransmitter Laboratory, under the leadership of Dr Gavin Lambert and the Neuropharmacology Laboratory, headed by Associate Professor Geoff Head.
Our research findings support the importance of psychological mechanisms and mental stress in heart disease and high blood pressure.

- Investigating the neurobiology of Obesity-Related Hypertension

This research aims to find a rational basis for the treatment of Obesity-Related Hypertension through ongoing studies into the way in which the development of obesity causes blood pressure elevation. Significant discoveries in this area include the finding that leptin, a hormone known to be secreted by fat tissue, is also produced in the human brain. Leptin release from the brain is markedly increased in human obesity, accounting for more than 25% of whole body leptin release. This discovery is highly important because leptin is an important regulator of body weight, making it the focus of a lot of attention in the fight against obesity worldwide.

- Investigating the role of the sympathetic nerves in renal hypertension

There is increasing evidence that sympathetic (stimulant) nerve activity is important in the development of hypertension, heart failure and renal failure. We have shown that the relative role of the renal sympathetic nerves in causing angiotensin-dependant hypertension diminishes with the development of the disease.

- Analysis of the neurobiology of 'neurogenic' essential hypertension

We have previously shown that this form of high blood pressure is initiated and sustained by overactivity of the sympathetic nervous system. In collaboration with Professor Graeme Jackson of the Brain Research Institute at Austin Health, we will apply functional Magnetic Resonance Imaging (MRI) methodology of selected regions of the brain to investigate neural mechanisms of this form of hypertension.

- Studying the neural pathophysiology of Postural tachycardia syndrome (POTS)

This common but enigmatic disorder is characterised by sufferers displaying a racing heart and blackouts upon standing. The cardiovascular neuroscience division is investigating the control mechanisms of the brain circuitry involved in POTS. Research focuses on the role of noradrenaline, one of the brain's 'messenger chemicals' or neurotransmitters. Significant discoveries in this area include the discovery of an epigenetic abnormality in the noradrenaline transporter gene in POTS patients, which may be the cause of the disorder.

- Exploring the mechanisms of heart risk in depressive illness

It has long been known that depressive illness materially increases heart risk, yet the precise mechanisms by which this occurs are yet to be fully explained. The cardiovascular neuroscience division has found that in approximately 40% of depressive patients, the level of activity in the sympathetic nerves of the heart is markedly elevated to the level seen in patients with terminal heart failure. This discovery may yield an explanation for how depressive illness constitutes a risk factor for heart disease.

- Investigating the neurophysiology of panic disorder

Abnormalities in the gene which codes for the noradrenaline transporter have also been described in patients suffering panic disorder. Our work adds to the growing knowledge of the mechanisms of how panic disorder contributes to increased cardiovascular risk. Part of this increased risk may be mediated by adrenaline, second neurotransmitter of the sympathetic nervous system. We have recently shown neurobiological abnormalities in adrenaline signalling in sympathetic nerves in patients suffering panic disorder. Work is ongoing investigating the effects on heart risk of two different treatments for panic disorder, cognitive behavioural therapy and selective serotonin reuptake inhibitor (SSRI) medication.

As well as having a global impact on the understanding of the interactions between the brain and the heart, the work of this division also extends to the local community. Sufferers of panic disorder are often unaware that they have a relatively common treatable illness, believing that they are either "going mad" or in grave danger of sudden death. By volunteering for studies such as those into the biology of panic disorder, sufferers in the local community often gain a better understanding of the causes of panic disorder, the level of heart risk (which in the majority of patients is, in fact, negligible) and potential ways to deal with the condition.

The cardiovascular neurosciences division has benefited from collaborations with The Institute For Molecular Bioscience in Queensland, studying novel peptides isolated from snake venom as a potential treatment for heart failure. We have established a Baker spin-off company, ElaCor, to develop these novel therapies.

INTERNATIONAL COLLABORATIONS

Associate Professor Geoff Head's lab hosted Dr Elena V Lukoshkova from the National Cardiology Research center in Moscow, Russia. Dr Gavin Lambert's lab hosted Dr Klemens Fellner from the Faculty of Mathematics, University of Vienna, Austria. International collaborators include Dr Phil Gold, a distinguished depressive illness researcher from the National Institutes of Health, USA, and Professor Mona Soreq, an international figure in neurogenetics, based at the Hebrew University, Jerusalem.

NEUROPHARMACOLOGY LABORATORY

Geoffrey A Head

Head
Geoffrey A Head - BSc (Hons), PhD

Senior Scientific
Dmitry N Mayorov - BSc (Hons), PhD

Professional & Technical
Sandra L Burke - BSc (Hons), MSc
Luisa La Greca - BBiol Sci (Hons)

Visiting Scientists
Dr Elena V Lukoshkova - National Cardiology Research Center, Moscow, Russia

Students
Nina Eikelis - (submitted PhD Dec 2004)
Scott Maxwell - (RMIT University, Pharmaceutical Sciences 40 week work placement student)

Research Projects

The role of the renal sympathetic nerves in angiotensin-dependent hypertension

S Burke, G Head

There is increasing evidence that sympathetic nerve activity is important in the development of hypertension, heart failure and renal failure. In this study, we assessed the role of the sympathetic nervous system and in particular, of the renal nerves, in maintaining high blood pressure in conscious rabbits made hypertensive by chronic infusion of angiotensin. We did this by comparing the size of the fall in blood pressure to ganglion blockade before and each week after beginning angiotensin treatment. After 5 weeks, we cut the renal nerves and repeated the experiment. We found that initially, the fall in blood pressure to ganglion blockade was similar in hypertensive and normal animals but after 2 weeks, the response to ganglion blockade was greater in the angiotensin group and by 4 weeks, the response was double that in untreated animals. When the renal nerves were cut, blood pressure did not change in either group. However, while the response to ganglion blockade was the same in the hypertensive rabbits, it increased in the untreated group. This study suggests that there is an important contribution to the hypertension by the sympathetic nervous system which increases with time. However, while the role of the kidney is important in normal animals, its contribution appears to be diminished in hypertensives when other beds may increase in importance.

Renal neuroeffector mechanisms in angiotensin dependent hypertension

S Burke, G Head, R Evans

High blood pressure is a leading risk factor for cardiovascular diseases which cause heart attacks and strokes. We have previously shown that the sympathetic nervous system is overactive in renovascular hypertension but the nerve activity to the kidney, which is an important organ in blood pressure regulation, is not elevated. This study aimed to determine how control of the sympathetic nervous system is altered in angiotensin dependent hypertension. In particular we examined whether the neural control of blood vessels, tubules and cells which release renin in the kidney is altered and if the altered regulation occurs within the kidney itself or in the brain. Rabbits were made hypertensive with chronic angiotensin treatment for 4-6 weeks. Using an anaesthetised rabbit preparation, we measured renal blood flow to both the medulla and cortex of the kidney and we obtained direct measurements of renin and noradrenaline produced by the kidney via a catheter in the renal vein. We stimulated the renal nerves by direct electrical stimulation of the postganglionic fibres and reflexly using low oxygen. There was increased production of renin and noradrenaline spillover by the kidney both in response to direct electrical stimulation and by reflex stimulation. Surprisingly, renin production was lower in animals with renovascular hypertension. Renal blood flow decreased with both types of nerve stimulation but while cortical blood flow was the same, medullary blood flow was overall lower in hypertensive than normal animals. Kidney function was less effective in angiotensin dependent hypertension but urine production was the same. These experiments indicate that the renal neuroeffector mechanism may not be enhanced in angiotensin dependent hypertension but may be diminished.

Role of reactive oxygen species in the brainstem in cardiovascular reactivity to emotional stress

D Mayorov, G Head

The excessive blood pressure (BP) responsiveness to psychoemotional stressors is a risk factor in the development of cardiovascular disorders such as hypertension and coronary heart disease. To date, remarkably little is known about mechanisms that modulate the cardiovascular susceptibility to emotional stress. Recently, we found that blockade of AT1 receptors in the rostral ventrolateral medulla (RVLM) abrogated the pressor effect of emotional stress in rabbits. Because superoxide has been shown to be an important intracellular mediator of actions of angiotensin II, in the present project, we examined the influence of superoxide dismutase (SOD) mimetics, tempol and tiron, in the RVLM on cardiovascular stress responses in conscious rabbits. Airjet stress evoked a sustained increase in blood pressure, tachycardia and renal sympathoactivation. Bilateral microinjections of tempol or tiron into RVLM did not alter resting cardiovascular parameters, but attenuated the pressor, sympathetic and tachycardiac response to stress. By contrast, 3-carbamoyl proxyl, which is structurally close to tempol but has a

lower superoxide scavenging activity, did not alter the stress response. Neither tempol nor tiron altered the sympathoexcitatory response to glutamate microinjections into the RVLM or to baroreceptor unloading. Microinjections of a nitric oxide synthase inhibitor L-NAME into the RVLM did not affect the stress response. Co-injections of tempol and L-NAME decreased the pressor response to stress and tempol attenuated the pressor response to microinjection of angiotensin II into RVLM, whereas L-NAME did not alter this response. These results suggest that SOD mimetics in the RVLM attenuate, partially via a nitric oxide-independent mechanism, the pressor effect of emotional stress in rabbits. Together with our previous studies, these results also indicate that superoxide is a key mediator of excitatory actions of angiotensin II in RVLM during acute stress.

Influence of angiotensin II in the dorsomedial hypothalamus on cardiovascular arousal caused by emotional stress and feeding

R De Matteo, G Head, D Mayorov

The dorsomedial hypothalamus (DMH) has been implicated in autonomic arousal caused by aversive stimuli and also in appetitive (positively motivated) feeding behavior. This study aimed to determine whether the DMH is important in feeding-induced autonomic arousal and, if so, whether distinct neurotransmitter systems in this region underlie autonomic arousal caused by stimuli of different emotional valence. Negative emotional (air-jet) stress and feeding elicited similar tachycardic and pressor responses in conscious rabbits. Bilateral microinjection of GABAA agonist muscimol into the DMH attenuated the pressor and tachycardic responses to air-jet and also evoked anorexia. Microinjection of an ionotropic glutamate receptor antagonist kynurenic acid into the DMH decreased the pressor and tachycardic responses to both stress and feeding. Conversely, local microinjection of a glutamate analog kainic acid elicited hypertension, tachycardia and activated feeding behavior. Microinjection of the angiotensin II (AT1) receptor antagonist candesartan into the DMH attenuated the pressor and tachycardic responses to stress by one third without altering feeding behavior or circulatory response to feeding. Candesartan also blocked the pressor response to local injection of angiotensin II. These results indicate that endogenous angiotensin II acting via AT1-receptors may specifically mediate, in the DMH, cardiovascular arousal caused by aversive stimuli. By contrast, local GABAergic and glutamatergic transmission appear to underlie autonomic arousal regardless of the emotional valence of the stimuli. These findings may have potential clinical implications, as they suggest that abnormalities in Ang –sensitive mechanisms within the DMH may play a role in increased autonomic responsiveness to psychological stressors, which is believed to be a risk factor for cardiovascular and other diseases.

Role of reactive oxygen species in the dorsomedial hypothalamus in cardiovascular response to stress and feeding in rabbits

R De Matteo, G Head, D Mayorov

Superoxide has been shown to be an important intracellular mediator of actions of angiotensin II. We found that blockade of angiotensin AT1 receptors in the dorsomedial hypothalamus (DMH) decreased autonomic arousal caused by emotional stress, but not feeding in rabbits. Because superoxide has been shown to be important as a intracellular mediator of the excitatory actions of angiotensin, in the present project, we examined the influence of superoxide dismutase (SOD) mimetics, tempol and tiron, in the DMH, on cardiovascular response to stress and feeding in conscious rabbits. Bilateral microinjection of tempol or tiron into the DMH attenuated the pressor and tachycardic responses to air-jet by 60%, without altering feeding behavior or circulatory response to feeding. By contrast, 3-carbamoyl proxyl, which is structurally close to tempol but has a lower superoxide scavenging activity, did not alter stress responses. Microinjection of vehicle did not alter cardiovascular responses to stress or to feeding. These results suggest that superoxide in the DMH may specifically mediate the cardiovascular arousal elicited by aversive stimulation. Conversely, superoxide appears to play little role in mediating cardiovascular arousal caused by feeding. These findings warrant further investigation, as, together with our previous data, they suggest that abnormalities in Ang –superoxide signalling pathways in the hypothalamus may be important in increasing cardiovascular reactivity to psychological stress.

The effects of S26939 and losartan on blood pressure in spontaneously hypertensive rats

R Rubenstein, L LaGreca, G Head

A relatively new class of antihypertensive agents such as rilmenidine, acts in the brain to lower blood pressure by reducing sympathetic nerve activity to beds such as the heart and kidney. We have previously shown that their central action involves mainly imidazoline receptors as well as alpha adrenoceptors. Newer generations of these drugs have been developed which are more selective for imidazoline receptors and have fewer side effects such as sedation, which were common in the older drugs like clonidine. One of these newly developed drugs is S26939 and the aim of the current study was to compare the effectiveness of acute administration of S26939 at lowering blood pressure, with that of the AT1-receptor antagonist, losartan. The experiments were conducted in adult spontaneously hypertensive rats (SHR) using radiotelemetry to record blood pressure over 24 hours, so that the animals could be studied in their home cages. Both S26939 and losartan dose dependently reduced blood pressure and S26939 lowered heart rate. S26939 was not as effective as losartan and its hypotensive effects were confined to the inactive period (day time) whereas losartan was effective over 24 hours. The study indicates that S26939 has

potential for use as an antihypertensive agent relatively free of side effects.

Effect of novel natriuretic peptide-like compounds on blood pressure and heart rate in conscious rabbits

G Head, S Maxwell

Conventional diuretic treatment for chronic heart failure is associated with activation of sympathetic nerves and the renin-angiotensin system and with renal impairment, which may have adverse long-term effects. Natriuretic peptides (NP) function as vasorelaxing hormones and therapy with them has benefits in hypertensive patients where it opposes the constrictor effects of angiotensin II, inhibits the sympathetic nervous system and lowers fluid retention. Novel natriuretic peptides have been isolated from the venom of the Western taipan snake. We determined the hypotensive effects of these synthetic peptides in conscious normotensive rabbits showing that one of the peptides lowered blood pressure as effectively as human atrial natriuretic peptide. These natriuretic peptides thus have potential as treatment for patients with conditions in which intravascular volume is increased, such as chronic heart failure and chronic and acute renal failure and may provide more appropriate therapy than at present.

Grants and other Funding

- NHMRC, Principal Research Fellowship. G Head.

- NHMRC, Project Grant, Central regulation of blood pressure: role of angiotensin and nitric oxide. G Head, D Mayorov.

- NHMRC, Project Grant, Neural control of renal function: functionally specific populations of sympathetic nerves. K Denton (Monash), G Head.

Commercially Funded Research Activities

- Servier Laboratories, Chronic effects of S26939 and rilmenidine on blood pressure in spontaneously hypertensive rats using radiotelemetry. G Head

- AusBio, Cardiovascular Profile Testing of MD960. G Head

- Mimotopes, Pharmacological actions of guanadino analogues in the anaesthetized rat, Kaye & Head

Presentations

- International Society for Hypertension, Brazil. D Mayorov, R de Matteo, G Head, Nitric oxide modulates sympathetic baroreflex in rostral ventrolateral medulla of the conscious rabbit.

- International Society for Hypertension, Brazil. D Mayorov, R de Matteo, G Head, Superoxide mediates excitatory actions of angiotensin II in the rostral ventrolateral medulla during emotional stress.

- International Society for Hypertension, Brazil. R de Matteo, G Head, D Mayorov, Angiotensin-superoxide pathway in the dorsomedial hypothalamus mediates the pressor response to emotional stress in rabbits.

- European Society of Cardiology, Germany. G Head, S Burke, Chronic rilmenidine reverses the baroreflex deficit and reduces sympathetic responses to stress in hypertensive rabbits.

- European Society of Cardiology, Germany. G Head, S Burke, Chronic rilmenidine reverses cardiac hypertrophy and the changes in renal haemodynamics associated with renovascular hypertension.

- 14th Meeting of the Sympathetic Nervous System Working Group, Germany. G Head, Effects of rilmenidine in 2K1C hypertensive rabbits.

- European Society for Hypertension, France. N Eikelis, R de Matteo, S Burke, M Esler, G Head, The effect of central leptin administration on cardiovascular responses in conscious rabbits fed a high-fat diet.

- Symposium on Central Cardiovascular Control - Future Directions, Sydney. G Head, Is sympathetic vasomotor discharge really elevated in hypertension?

- Symposium on Central Cardiovascular Control - Future Directions, Sydney. D Mayorov, Little functional cross-talk between NO and superoxide signalling in the RVLM in vivo : what does it mean?

- Australian Neuroscience Meeting, Melbourne. D Mayorov, R de Matteo, G Head, Influence of nitric oxide on sympathetic baroreflex in the rostral ventrolateral medulla of the conscious rabbit.

- Australian Neuroscience Meeting, Melbourne. D Mayorov, R de Matteo, G Head, Role of superoxide and nitric oxide in sympathoexcitatory action of brain angiotensin during emotional stress.

- Australian Health and Medical Research Congress, Sydney. D Mayorov, G Head, R de Matteo, AT1 receptors in the dorsomedial hypothalamus and brainstem mediate cardiovascular arousal caused by emotional stress but not feeding.

- Australian Health and Medical Research Congress, Sydney. S Burke, G Head, Greater sympathetic contribution to chronic angiotensin II hypertension is not dependent on renal nerves.

- Australian Health and Medical Research Congress, Sydney. G Head, S Burke, Chronic rilmenidine in rabbit renovascular hypertension: effects on responses to acute hypotension and saline loading.

- Neural, hormonal and renal interactions in long-term blood pressure control, India. S Fitzgerald, H Parkington, B Kemp, G Head, R Evans, Role of NO in arterial pressure control during diabetes in mice.

HUMAN NEUROTRANSMITTER LABORATORY

Gavin Lambert

Head
Gavin Lambert - PhD

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Students
Jake Anderson - Honours (Monash)
David Barton - MBBS
Tye Dawood - PhD (Monash)
Nina Eikelis - BSc (Hon)

Research Projects

The neurobiology of essential hypertension

M Schlaich, E Lambert, D Kaye, G Lambert, F Socratous, E Hotchkin, G Jennings, M Esler

Our comprehensive examination of the neurobiology of hypertension has continued through 2004. Available evidence indicates that in a substantial proportion of patients essential hypertension is neurogenic, with documentation of high rates of spillover of noradrenaline from the heart and kidneys and increased firing of muscle (vasoconstrictor) sympathetic nerves. Although this increased cardiac and renal spillover of noradrenaline is attributable at least in part to increased sympathetic nerve firing rates, our recent data indicates that there may be some impairment of neuronal noradrenaline reuptake after its release from sympathetic nerves. In addition, using hand vein biopsies obtained from volunteers, we are examining the hypothesis that there might be an increase in the density of sympathetic innervation in human hypertension. Facilitation of neuronal noradrenaline release by adrenaline released from sympathetic nerves as a cotransmitter, and impairment of neuronal noradrenaline reuptake after its release from sympathetic nerves are also possibilities under investigation in this project.

Obesity and obesity-related hypertension

E Lambert, N Eikelis, E Hotchkin, M Schlaich, J Hastings, G Lambert

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. It is now becoming clear from our own observations and those of others that the link between obesity and hypertension involves sympathetic nerves. The impetus for investigating sympathetic activity in obesity stems from the putative role played by the sympathetic nervous system both in the genesis of obesity and the cardiovascular complications associated with obesity. The sympathetic nervous system is important in virtually all of the components of daily energy expenditure, including resting metabolic rate, energy expenditure associated with physical activity, the thermic effect of food, cold-induced thermogenesis, and thermogenesis related to daily stimulants including caffeine and nicotine. Indeed, it is well known that increased sympathetic nerve activity, if sustained, contributes to the development of hypertension.
Our research plans in obesity are driven by these facts, and in their content draw on the new biology of leptin, a 167 amino acid protein product of the ob gene produced by adipose tissue that has been identified as a blood borne factor regulating appetite. Also unclear are the predisposing factors, genetic or otherwise, which dictate that obesity leads to hypertension; perhaps 50% of the obese do not have hypertension. We are investigating these matters further.

The neurobiology of panic disorder

C Pier, M Alvarenga, E Lambert, J Richards, D Barton, F Socratous, M Esler

Until recently panic disorder was considered distressing and disabling but not a risk to life. However, it is now clear that there is a 3-6 fold increased risk of myocardial infarction and sudden death in patients with panic disorder.
The cause is not known, but cardiac sympathetic activation during a panic attack, which can induce ventricular tachyarrhythmias, and activation of platelets by high plasma catecholamine concentrations leading to thrombogenesis, are candidates.

Our observations also incriminate coronary artery spasm and pathological release of adrenaline from the heart as a sympathetic cotransmitter. Delineating the CNS neurochemical mechanisms underlying panic disorder (our recent finding is that activation of CNS serotonergic systems is the neurochemical "signature" of panic disorder), quantifying the cardiac sympathetic outflow during panic attacks, examining the cardiac and sympathetic baroreflex and establishing whether platelet activation is present, will indicate which pharmacological measures for prophylaxis of sudden death should be considered. We are continuing to build on our extensive studies of the neurobiology of panic disorder in this way, and in addition comparing the effects of therapeutic intervention with selective serotonin reuptake blockers and cognitive behavioural therapy.

Depressive illness and the heart

D Barton, T Dawood, E Lambert, E Hotchkin, F Socratous, G Lambert

Recent reports demonstrate that patients with depressive illness are at an increased risk of developing coronary heart disease. This increase is independent of conventional risk factors, and is so marked that advisory bodies have concluded that depressive illness is a proven cause of coronary heart disease, ranking with established risk factors (high blood pressure, cigarette smoking and lipid disorders) in terms of its impact. The mechanism of increased cardiac risk in depressive illness is uncertain, but activation of the peripheral nervous system is likely to be of prime importance. It is likely that related neural mechanisms are of importance in determining the depression and the development of increased cardiac disease in patients with depressive illness. Clearly, identifying the underlying mechanisms responsible and testing whether therapeutic interventions can reduce cardiac risk will be an important step forward in alleviating the burden of depressive illness on the community.

In this study we are measuring the nervous activity in the heart and blood vessel wall muscle (if you activate these nerves the blood vessels get narrower), the release of neurotransmitters from the brain and the brain's regulation of blood pressure and heart rate in patients with depressive illness. Patients are being examined soon after diagnosis and then following treatment with a serotonin specific reuptake inhibitor (a commonly used, effective antidepressant medication). We have recruited over 20 subjects and have directly measured the rate of release of noradrenaline (a chemical messenger released from the nerves) from the heart. Our observations indicate that in unmedicated patients with depression the nervous activity of the heart and blood vessels follows a bimodal pattern ie. in some patients the values are very high and in others very low – very few are "normal". Understanding the reason why this difference occurs presents us an exciting challenge. Interestingly, pharmacological treatment of depression is associated with a reduction in sympathetic nervous activity. Moreover, the regulation of blood pressure and heart rate in patients with depressive illness is significantly modified by therapy. Impaired regulation of blood pressure and heart rate has been directly linked to incidence rates for acute cardiac events in conditions such as hypertension, diabetes and myocardial infarction. These insights into the mechanisms responsible for generating cardiac risk may pave the way for novel therapeutic strategies to be administered in order to modify cardiac risk.

Grants and other Funding

- Australian Rotary Health Research Fund, The neurobiology of depressive illness: causes and consequences of altered brain monoaminergic function. G Lambert, D Barton, D Kaye, M Esler.

- NHMRC Program Grant, Heart failure and its antecedents. G Jennings, A Bobik, A Dart, M Esler, D Kaye.

- NHMRC Project Grant, Depressive illness and the heart: Identifying the relation between affective disorders and coronary heart disease. G Lambert, D Barton, D Haikerwal, E Lambert.

- NHMRC Project Grant, Panic disorder: Neurobiology and mechanisms of cardiac risks. M Esler, E Lambert , J Richards.

- National Heart Foundation, Affective disorders and their association with the cardiovascular system. T Dawood.

Commercially Funded Research Activities

- Solvay Pharmaceutical Company, Investigator initiated drug trial. M Esler, E Lambert.

Presentations

- Collegium Internationale Neuro-Psychopharmacologicum Congress, France. T Dawood, Sympathoadrenal activation in depression pre and post-treatment.

- European Society of Hypertension, France. N Eikelis, The effect of central leptin administration on cardiovascular

responses in conscious rabbits fed a high-fat diet.

- Federation of American Society of Experimental Biology meeting, USA. M Esler, Sympathetic nervous system function in age-associated clinical diseases.

- Danish Cardiovascular Research Academy and Faculty of Health Sciences European Union PhD Course on Integrative Cardiovascular Control, Denmark. M Esler, Invited as course teacher.

- 17th Annual Meeting of the Japan Microneurography Society, Japan. M Esler, Microneurography and measurement of noradrenaline spillover: complementary methods for investigating the neurology of obesity.
European Society of Hypertension, France. M Esler, The sympathetic nervous system, systolic blood pressure and beyond: New clinical data with Eprosartan.

- Collegium Internationale Neuro-Psychopharmacologicum Congress, France. M Esler, Autonomic nervous system in anxiety disorders.

- International Society of Heart Research Satellite Meeting, Victoria. M Esler, Neural control of the heart in aging and heart failure.

- Visiting Professor, Royal Perth Hospital, Western Australia. M Esler.

- St. Vincent's Hospital Campus Research Day, New South Wales. M Esler.

- Institute for International Health and Baker Heart Research Institute Combined Annual Research Meeting, Queensland. M Esler, Sympathetic nervous activation with "healthy" aging; Contribution to the development of cardiovascular disease?

- Festschrift of Professor Graeme Smith, Vicoria. M Esler, The neurobiology of psychosomatic heart disease.

- 2nd Australian Health and Medical Research Congress (AHMRC), New South Wales. L Guo, An epigenetic mechanism for the phenotype of faulty sympathetic neuronal noradrenaline reuptake in essential hypertension.

- Geelong Hospital invited lecture, Victoria. G Lambert, Depression and panic disorder: Novel risk factors for cardiovascular disease.

- Centre for Reproduction and Development seminar, Victoria. G Lambert, Towards and understanding of psychosomatic heart disease.

- Mental Health Research Institute Open Forum, Victoria. G Lambert, Towards and understanding of psychosomatic heart disease.

- Neurosciences Victoria seminar series, Victoria. G Lambert, Psychosomatic heart disease.

- Stress, Depression and Heart Disease, a joint initiative by the Australian National Heart Foundation and Caulfield Cardiac Rehabilitation Unit, Victoria. G Lambert, Depression and the heart.

- Federation of American Society of Experimental Biology meeting, USA. G Lambert, Neural mechanisms in cardiovascular regulation.

- European Society of Hypertension, France. E Lambert, Reduced sympathoneural responses to the cold pressor test in individuals with essential hypertension and in those genetically predisposed to hypertension. No support for the "pressor reactor" hypothesis of hypertension development.

- National Seniors Association, Victoria. G Wiesner, Health risks of obesity

VESSELS - VASCULAR DIVISION

Mark Cooper

The Vascular Division is a diverse group of laboratories with three main subdivisions. All are engaged in the exploration and identification of the causes, processes, effects and new treatments of vascular disease.

The Vascular Biology group has a very strong interest not only in atherosclerosis but particularly in how it relates to the increasing problem of diabetes. A major focus of the research of this group extends to exploring atherosclerosis and vascular changes caused by genes, hormones, diet, exercise, ageing, high blood pressure and drugs. Atherosclerosis is the formation of lipid fatty deposits in the vessel wall. When this occurs in the coronary vessels it leads to heart attacks, when it occurs in the brain it can lead to stroke. Deposition of cholesterol, the major component of the lipid plaques in the vessel is a result of an imbalance between delivery of cholesterol to the vessels and removal of excess cholesterol. Preventing the formation of cholesterol and therefore delivery of cholesterol to tissues has been the way that the group of drugs known as statins work to successfully lower cholesterol and prevent heart attacks.

The Lipoprotein and Atherosclerosis laboratory is aimed at determining pathways involved in removal of cholesterol from the vessel wall and looking for ways of enhancing its removal. This would provide an alternative to statin therapy or an additional drug to treat atherosclerosis.

Similarly the Cell Biology group are also trying to understand the development of the fatty lesions in blood vessels and the processes responsible for the progression of the lesions to the stage where they rupture and cause heart attack or stroke.

At a clinical level, the Cardiovascular Nutrition group is focussing on investigating nutrition and food related strategies that may contribute to cardiovascular health. This group has performed a study in elite athletes which provides strong clues about the mechanisms through which physical activity raises blood high density lipoprotein (good cholesterol) levels.

Also at a clinical level the Experimental and Human Vascular Biology group are looking at the relationship between lipids, the endothelium (vessel lining) and atherosclerosis.

The Clinical Physiology section has two major areas of interest. Diabetes is a major health problem, particularly late onset of Type II Diabetes. It is well known that exercise improves blood glucose control in diabetes and their laboratory has now unravelled the mechanism by which exercise improves glucose control. Understanding this means that we may be able to develop drugs that mimic the action of exercise. The second area that this laboratory is involved with is the compliance or stiffness of large arteries. As we age our arteries get stiff and it is now known that this is a risk factor for heart disease. This group has recently demonstrated that artery stiffness aggravates coronary artery disease leading to heart attacks. They have also shown that there are certain genetic factors, particularly variations in gene coding for the structural components or building blocks of artery walls that increase the risk of large artery stiffening leading to rises in systolic blood pressure and coronary artery disease.

The third grouping in the division studies diabetes and its complication. This is headed by Professor Mark Cooper who has expanded his large program on diabetic complications. With the growing number of obese people and the lack of exercise, Type II diabetes which usually occurs in middle age and is not due to lack of insulin, is an important public health problem throughout the world. However most diabetics, both Type I and Type II, nowadays do not die from the metabolic abnormalities of diabetes but from cardiovascular and importantly atherosclerotic disease. The group has a longstanding international reputation in diabetic nephropathy which leads to kidney failure defining treatment strategies to slow the progression of diabetic renal disease. The group also explores why diabetes leads to accelerated atherosclerosis and why 70% of people with diabetes die from cardiovascular disease, mainly heart attacks and strokes. In a new model of experimental diabetes associated atherosclerosis, which develops spontaneous fatty streaks and plaques in the vessel, they have identified that specific treatments useful for the treatment of kidney diseases are also helpful in treating or preventing atherosclerosis in diabetes.

The high sugar levels in diabetes lead to specific chemical irreversible reactions between the excess sugar and proteins such as haemoglobin and other structural proteins. This process is called advanced glycation. In diabetes these proteins accumulate in may sites causing disruption of normal tissue structure and function. The Glycation and Complications group has shown that these molecules not only cause structural changes but activate many harmful processes in the heart, kidney and blood vessels. The group has recently identified the role of a new treatment which dissolves these abnormal and sticky proteins and are currently translating their experimental findings into man, specifically Type I diabetic subjects with very early kidney disease.

This research into diabetes is part of a program recently renewed by the Juvenile Diabetes Research Foundation International, and the National Health & Medical Research Council of Australia. Furthermore, this group was recently awarded a large international grant as a part of a consortium involving the Albert Einstein College of Medicine in New York and the University of Heidelberg in Germany.

HUMAN VASCULAR BIOLOGY LABORATORY

Stephen J Duffy

Head
Stephen J. Duffy - MB, BS (Hons), PhD, FRACP, MRCP

Professional & Technical
Lovisa Dousha - BSc (Hons) – from January, 2005
Jessica Ziolkowski - BSc (Hons) – until 11.2.2005

PhD Students
Swati Mukherjee - MB, BS
Darren Henstridge - BSc (Hons)

Research Projects for 2004
Relationship of Iron Status to Oxidative Stress in vivo, Nitric Oxide Bioactivity and Coronary Artery Disease Activity

Project 1:
Investigators: J. Ziolkowski, S. Mukherjee, L. Dousha, D.K. Vizi, N.C. Gollogly, A.M. Dart & S.J. Duffy

Background: Patients with high total-body iron levels may be at higher risk for the development of atherosclerosis and coronary artery disease. In animal models of atherosclerosis, iron has been shown to contribute to the progression of disease development. Endothelial-derived nitric oxide (EDNO) is an important anti-atherosclerotic molecule, and its activity is decreased in patients with coronary artery disease. In this study we are testing the hypothesis that acutely increasing extracellular iron concentration will attenuate EDNO bioactivity in the peripheral vasculature of healthy volunteers and patients with coronary artery disease.

Results & Conclusions: To date, we have tested the forearm vasodilator response of a large group of healthy volunteers to the endothelium-dependent and -independent agonists acetylcholine and sodium nitroprusside, respectively, before and after intra-arterial infusion of a parenteral iron preparation. Surprisingly, our data show no adverse effect or iron on vasodilator function in these healthy volunteers. We have collected serum samples from the volunteers and are currently analysing these for various measures of oxidative stress. These will allow us to determine the level of oxidative stress induced by the iron infusion and to what extent we are affecting the levels of EDNO bioactivity. Additionally, we are repeating these studies in a group of patients known to have increased oxidative stress: patients with coronary artery disease.

Project 2:
Investigators: S. Mukherjee, A.J. White, B.A. Kingwell, A. Walton, L. Dousha, A.M. Dart & S.J. Duffy

Background: Reactive oxygen species have been implicated in the pathogenesis of atherosclerosis. Redox-active iron increases production of reactive oxygen species (via Fenton chemistry) and causes lipid peroxidation. F2-isoprostanes are a reliable method for assessment of oxidant stress in vivo. We aimed to determine the relationship of these markers of oxidative stress in coronary blood samples to clinical presentation and plaque characteristics. Methods: Thus far we have recruited 36 patients undergoing percutaneous coronary intervention (PCI; age 66±11 years, mean±SD, 30 male). Trans-lesional blood samples were collected before and after PCI from aorta and distal coronary artery. Intravascular ultrasound was performed prior to revascularization. Clinical presentation was classified by Braunwald criteria (16 stable, 20 unstable).

Results: Aortic high-sensitivity C-reactive protein (hsCRP) was higher in unstable patients (22.4±38.3 vs. 4.3±4.7mg/L, p=0.01). There was a trans-lesional gradient of ferritin at 3 and 10 minutes after PCI (17.9±30.7µg/L, p=0.003 and 17.3±24.6µg/L, p=0.0004, respectively). Ferritin only weakly correlated with hsCRP (r=0.32, p=0.06). There was a trans-lesional gradient of F2-isoprostanes at baseline (589±146pmol/L proximally vs. 645±170pmol/L distally, p=0.036), with a similar trend 3 minutes after PCI. The F2-isoprostane level distal to the lesion 3 minutes after PCI correlated with the plaque area at maximum stenosis (r=0.51, p=0.038) and with troponin-I (r=0.47, p=0.02).

Conclusions: These data suggest that iron stores and markers of oxidative stress are increased in coronary atherosclerotic plaque, and that these are released with plaque disruption. Furthermore, markers of oxidative stress relate to plaque burden. This supports the concept that iron stores and oxidative stress influence coronary atherosclerosis.

The Role of Non-prostanoid, Non-nitric oxide, Endothelium Derived Factors in Patients with Cardiovascular Disease

Investigators: J. Ziolkowski, J.P.F. Chin-Dusting, S. Mukherjee, L. Dousha, N.C. Gollogly, D.K. Vizi, J. McPherson, M. Ralph & S.J. Duffy

Background: We have previously demonstrated that endothelium-derived vasodilators such as nitric oxide and prostacyclin contribute to metabolic vasodilation in skeletal muscle and coronary artery vasculature. In vascular smooth muscle cells, efflux of K+

through large-conductance Ca-sensitive K+-channels and ATP-sensitive K+-channels results in hyperpolarization, relaxation and consequent vasodilation.

Methods: In this study we are testing the hypothesis that activation of Ca-dependent K+-channels will contribute to endothelium-dependent metabolic and agonist-mediated vasodilation of skeletal muscle resistance vasculature, and that this contribution will be greater in disease states associated with reduced bioavailability of nitric oxide.
Results & Conclusions: We have shown that the forearm vasodilator response to acetylcholine was impaired in patients with coronary artery disease (CAD) compared to that of healthy volunteers. Administration of tetraethylammonium (TEA), a compound that selectively blocks Ca-sensitive K+-channels, decreased resting blood flow in healthy volunteers but not in patients with CAD. Interestingly, TEA had no effect on the volume of blood "repaid" over one or 5 minutes after exercise in either group, despite adjustment for changes in baseline. These data suggest that tonic activation of Ca-dependent K+-channels contributes to resting blood flow in skeletal muscle vasculature in health, but not in patients with CAD.

We are currently investigating the contribution of Ca-dependent K+-channels in the absence of NO and prostacyclin, two other important vasodilators that can cause vascular smooth muscle cell hyperpolarization. Thus far, TEA has not attenuated the vasodilator response to acetylcholine in either healthy controls or patients with CAD. Combined inhibition of NO and prostanoids with NG-monomethyl-L-arginine and aspirin, respectively, attenuated the response to acetylcholine in healthy controls (from 20.1±9.2 to 14.6±9.9ml/100ml/min for the highest dose, p=0.01), but co-infusion of TEA did not further reduce the response to acetylcholine (14.4±7.4ml/100ml/min, p=0.63). Additionally, TEA alone did not attenuate the vasodilator response to bradykinin. These data suggest that activation of Ca-dependent-K+-channels does not contribute to agonist-induced vasodilation in the skeletal muscle vasculature of humans.

Nitric Oxide and Oxidant Stress in Human Hypertension

Investigators: S. Mukherjee, J. Ziolkowski, L. Dousha & S.J. Duffy

In this NHMRC funded (Project Grant) study we are testing the hypotheses that hypertension in humans, including low-renin hypertension, is associated with increased oxidative stress, and that amelioration of this oxidative stress with the antioxidant ascorbic acid will lower blood pressure by increasing nitric oxide bioactivity.

As this is a double-blind, randomised, placebo-controlled study, we will not have data available until the completion of the trial, but good progress is currently being made on this project.

Contraction-mediated Glucose Uptake as a Therapeutic Target in Type 2 Diabetes Mellitus

Investigators: D. Henstridge, S.J. Duffy & B.A. Kingwell

In this NHMRC funded (Project Grant) study we are testing the hypothesis that a separate pathway to insulin-mediated glucose uptake can be utilised in patients with type 2 diabetes mellitus to improve glycaemic control. In type 2 diabetics there is insulin resistance, but contraction- (or exercise-) mediated glucose uptake appears to be of increased importance. Our preliminary data, we have evidence that nitric oxide may stimulate this pathway and we are currently testing this as a therapeutic tool in a clinical trial.

Prevention of the No-reflow Phenomenon in Acute Coronary Syndromes

Investigators: A.J. Taylor, H. Thomson, R. Reddy, S. Mukherjee, M. Kelly & S.J. Duffy

Background: Defined angiographically, the "no-reflow" phenomenon manifests as an acute reduction in coronary blood flow in the absence of epicardial vessel obstruction. This may result in (further) myocardial infarction if it persists. This occurs in 30% to 40% of cases of acute myocardial infarction despite no residual stenosis at the original site of coronary occlusion after percutaneous transluminal coronary angioplasty (PTCA) and stenting, and is associated with a poor prognosis. This clinical project follows on from some of the experimental work at the Baker by Professor Bobik and Dr. Taylor.

The aim of this prospective, randomised study is to determine:
- Whether the vasodilators sodium nitroprusside (SNP) and verapamil will improve coronary blood flow, coronary flow reserve and reduce myocardial infarct size in patients with myocardial infarction.
We hypothesize that both of these strategies will prevent the no-reflow phenomenon in patients with myocardial infarction. In the interim, we have performed a retrospective study of the use of SNP in the Alfred Hospital Catheterization Laboratories.

Methods and Results: In this part of the study, we retrospectively reviewed the cases of 20 patients treated with SNP for no/slow-reflow between October 2002 and February 2005. The mean age was 70±9 (SD) years; 12 were male. Sixteen were undergoing PCI for acute or recent MI, 3 were being treated for unstable angina, and one had stable angina. The target vessel was a

saphenous vein graft in three of the 4 without acute MI, including the patient with stable angina. Ten of the remaining (native) vessels were the LAD, with the RCA and circumflex representing 4 and 3 vessels, respectively. The Thrombolysis In Myocardial Infarction (TIMI) flow after stenting (in all patients) was 1.2±0.8, which improved to 2.4±0.9 after SNP, $p<0.0001$. The mean dose given was 556±218 mg. In the 16 MI patients the TIMI flow increased from 1.0±0.7 to 2.3±1.0 after SNP, $p<0.0001$. Conclusions: These data suggest that SNP is an effective and safe treatment for no/slow-reflow in the setting of PCI for MI. Prospective, randomised studies will be required before this therapy can be recommended generally.

Melbourne Interventional Group Database

Investigators: S.J. Duffy, J. Shaw, D. Clark, C. Reid & A. Ajani

This is a co-operative database of percutaneous revascularization procedures that includes most of the Victorian public hospitals that perform coronary interventions. Our ultimate aim (with respect to the database) is to obtain short- & long-term outcomes of coronary interventions for all patients in the state, though we are currently obtaining a representative sample. A number of abstracts with preliminary data have been submitted to the Cardiac Society of Australia & NZ annual scientific meeting for 2005. An additional aim is to provide a framework to complete interventional/clinical trials.

Grants, fellowships and other funding

1. National Health & Medical Research Council of Australia Project Grant
Sole Chief Investigator: Stephen J. Duffy
"Nitric oxide and oxidant stress in hypertension"

2. National Health & Medical Research Council of Australia Project Grant
Chief Investigators: Bronwyn A. Kingwell & Stephen J. Duffy
"A novel mechanism for manipulation of peripheral glucose utilization in patients with type 2 diabetes mellitus"

3. National Health & Medical Research Council of Australia Career Development Award
Research Fellowship: Stephen J. Duffy

4. Diabetes Australia Research Trust
Chief Investigators: Bronwyn A. Kingwell & Stephen J. Duffy
"A novel mechanism by which HDL cholesterol may modulate glucose and fat metabolism"

5. Collier Charitable Fund Grant
Sole Chief Investigator: Stephen J. Duffy
Equipment grant to purchase the Radi Analyzer and Pressure Wires for measurement of myocardial fractional flow reserve

Publications 2004

- Widlansky, M.E., Biegelsen, E.S., Hamburg, N.M., Duffy, S.J., Keaney, J.F., Jr., Vita, J.A.
Coronary endothelial dysfunction is not rapidly reversible with ascorbic acid.
Free Radical Biology and Medicine, 2004; 36: 123-30. Impact factor: 5.1

- Widlansky, M.E., Duffy, S.J., Hamburg, N.M., Gokce N., Warden, B.A., Wiseman, S., Keaney, J.F., Jr., Frei, B., Vita, J.A.
Effects of black tea consumption on plasma catechins and markers of oxidative stress and inflammation in patients with coronary artery disease.
Free Radical Biology and Medicine, 2005: 38: 499-506. Impact factor: 5.1

- White, A.J., Walton, A.S., Duffy, S.J., Dart, A.M., Jennings, G.L., Drew, B.G., Kingwell, B.A.
A translesional concentration gradient of matrix metalloproteinase-2 (gelatinase A) but not metalloproteinase-3 (stromelysin-1) exists across stenotic coronary plaques.
Heart, Lung and Circulation, 2004; 13 (Supplement 2): S99.

- Hengel, C.L., Duffy, S.J., Kaye, D.M.
Decision making in patients admitted post out-of-hospital cardiac arrest.
Heart, Lung and Circulation, 2004; 13 (Supplement 2): S105.

- Ziolkowski, J., Chin-Dusting, J.P.F., Gollogly, N.C., Vizi, D.K., Mukherjee, S., McPherson, J., Ralph, M., Duffy, S.J.
Contribution of endothelium-dependent hyperpolarization (via activation of Ca-dependent K+-channels) to metabolic vasodilation in the human forearm.
Heart, Lung and Circulation, 2004; 13 (Supplement 2): S107-8.

- Kingwell, B.A., Henstridge, D., Drew, B.G., McConell, G.K., Duffy, S.J.
The nitric oxide donor, sodium nitroprusside increases glucose uptake across the leg in patients with type 2 diabetes mellitus.
Heart, Lung and Circulation, 2004; 13 (Supplement 2): S131.

CARDIOVASCULAR NUTRITION LABORATORY

Paul Nestel

Head
Paul Nestel - AO, MD, FTSE, FRACP, FAHA

Senior Scientific
Nora Straznicky - B Pharm, PhD, MPh

Professional & Technical
Marja Cehun - BEd, RN
Andriana Chronopoulos - BSc (Hons)

Visiting Scientists
Lei Zhang - MD, Peoples Republic of China
Akihiko Fujii - BS, MS Pharmacy
Tohoku University, Japan

Research Projects

Isoflavone Research

P Nestel, M Cehun, A Chronopoulos

Following the successful demonstration in a series of studies concluding in early 2003 that isolated isoflavones (from red clover) reduce both arterial stiffness and the concentration of circulating adhesion molecule VCAM-1, we have concentrated on the potential of isoflavone metabolites and new chemical entities to modify atherogenic processes. Isoflavones are mostly consumed through soybeans and epidemiologically high intakes have been associated with less cardiovascular disease.

A further trial, to be completed in 2005 is measuring pulse wave velocity (measure of arterial stiffness) and metabolic parameters in 24 subjects with the metabolic syndrome in response to a novel isoflavone trans-tetra hydrodaidzein. This compound was chosen on the basis of its in vivo antioxidant and anti-atherogenic efficacy in our pre-clinical studies during 2003. Further bioassays of the effects of novel isoflavone analogues obtained from a pharmaceutical company on adhesion molecule expression in addition to antioxidant effects have been carried out. One highly active compound has been selected for further trial in rabbits with damaged carotid artery (with A. Bobik).

Studies on HDL Metabolism

P Nestel, M Cehun, A Chronopoulos

The effects of a new statin, rosuvastatin on HDL metabolism are being studied in subjects with the metabolic syndrome (insulin resistance, visceral adiposity, dyslipdaemia, hypertension). A randomised, double-blind, placebo controlled, cross-over trial is being carried out at this Institute (Cardiovascular Nutrition and Lipoprotein Laboratories) and also in collaboration with Professor G Watts at Royal Perth Hospital. Measurements focus on in vivo and in vitro components of reverse cholesterol transport, the mechanism through which cellular cholesterol is removed from cells for eventual excretion from the body. The study will complete in early 2005.

Complications of the Metabolic Syndrome

P Nestel, N Straznicky, M Cehun

The metabolic syndrome, components of which include visceral adiposity, hypertension, insulin resistance and dyslipidaemia, is a rapidly increasing disorder giving rise to resurgence of cardiovascular disease and Type 2 diabetes. Overactivity of the sympathetic nervous system in these subjects is a probable outcome of obesity and a likely contributor to hypertension. In collaboration with Drs Gavin and Elizabeth Lambert and Professor Murray Esler, we have completed a trial of the effects of weight reduction on the various components of the metabolic syndrome. Sympathetic nervous system activity measured by microneurography and whole-body noradrenaline production that were increased initially in these 22 subjects declined significantly with weight loss. Additionally, insulin sensitivity and plasma lipid profile improved and correlated with reduced sympathetic overactivity.
The study is progressing with the inclusion in 2005 of an exercise-weight reduction arm.

Effects of Dairy Fats on Plasma Lipids and other Cardiovascular Risk Factors

P Nestel, A Chronopoulos, M Cehun

Whereas butter fat consumption correlates strongly with coronary heart disease mortality across populations, such an association is not seen as clearly for cheese consumption. France and Switzerland, high consumers of cheese, tend to have less coronary deaths relative to saturated fat consumption. The possibility that the fermentation process involved in cheese making diminishes the almost predictable increase in plasma cholesterol that is found with consumption of butter was tested in a cross-over trial of the two dairy foods in 24 mildly hypercholesterolaemic subjects. Similar amounts of milk fats from either cheese or butter were compared within a standard cholesterol-lowering diet. Whereas butter fat raised plasma lipids especially low density lipoprotein cholesterol significantly and

substantially, the same amount of milk fat in cheese raised LDL minimally and not significantly, posing the possibility that advice regarding consumption of cheese may need revising.

Effects of novel edible oils on atherogenesis in mice

P.Nestel, A. Fujii
In collaboration with T. Allen (Atherosclerosis Laboratory)

Two novel oils, diacylglycerol (DAG) and conjugated linoleic acid (CLA) have attracted interest in nutrition research. Consuming DAG leads to lower increments in plasma triglyceride than an equivalent mass of oil as conventional triglyceride (TAG). That this may lead to less atherosclerosis and diminished induction of atherogenic genes is being tested in the apoE knock-out diabetic mouse in a comparison with TAG. CLA has been shown to suppress inflammatory genes and is being tested in the same mouse model with appropriate controls.

Commercially Funded Research Activities

- Novogen Ltd, Isoflavone Research Project. P Nestel, M Cehun, A Chronopoulos.

- Dairy Australia, Cheese versus butter on plasma cholesterol concentration. P Nestel, M Cehun, A Chronopoulos.

- Dairy Australia, Effect of Conjugated linoleic acid on atherosclerosis in mice. P Nestel, T Allen, A Fujii.

- Kao Corporation, Japan, Comparison of Diacylglycerol and triacylglycerol on atherosclerosis in mice. P Nestel, T Allen, A Fujii.

- AstraZeneca, Effect of Rosuvastatin on HDL mediated reverse cholesterol transport. P Nestel, D Sviridov.

Presentations

- P Nestel gave the following presentations that were either original research lectures or invited lectures that also included research findings.

- Invited Lectures:
Australian Atherosclerosis Society
International Clinical Nutrition Congress
International Life Sciences Conference
Pfizer Cardiovascular Symposium (workshop).

- Research presentations:
European Atherosclerosis Society Congress, Spain.
Drugs Affecting Lipid Metabolism, Italy.

DIABETIC ATHEROSCLEROSIS LABORATORY

Terri Allen

Head
Terri Allen - PhD

Professional & Technical
Philip Koh - PhD
Sandra Miljavec - DipApplSci
Gavin Langmaid
Sheree Purcell

Visiting Scientists
Assoc Prof Richard O'Brien - Monash Medical Centre, Melbourne

Students
Daniella Brasacchio - Honours (Monash) grad 2004
Anna Calkin - PhD (Monash) ongoing
Belinda Davis - PhD (Melbourne) grad 2004
Louis Teo Loon Yee - AMS (Monash) grad 2004

Research Projects

Pyridoxamine inhibits formation of advanced glycation end products decreasing atherosclerotic plaque formation in diabetic apoE KO mice
D Brassachio, J Forbes, A Calkin, M Cooper, T Allen
The role of the AGE and ALE inhibitor pyridoxamine was investigated in our model of type 1 diabetic atherosclerosis. Pyridoxamine was found to attenuate the atherosclerosis after 10 weeks of treatment and 20 weeks of diabetes. This occurred in the setting of reduced blood pressure, inhibition of AGE formation, decreased RAGE expression and attenuated expression of the growth factors TGFß1 and VEGF. Pyridoxamine may be a primary agent for diabetes-associated atherosclerosis. Further investigations are required of the mechanisms are now required.

Imatinib attenuates diabetes-associated atherosclerosis
M Lassila, T Allen, Z Cao, V Thallas, K Jandeleit-Dahm, R Candido, M Cooper
The novel blocker of PDGF imatinib was investigated in diabetic atherosclerosis. Diabetes was associated with an increase in PDGF gene expression in aorta of diabetic rats when measured by real-time RTPCR as well as increase protein expression determined by immunohistochemistry. Imatinib was able to reduce the formation of plaque as well as this increase in gene and protein expression. This may prove to be a novel therapy for diabetic atherosclerosis.

Rosiglitazone attenuates atherosclerosis in a model of insulin deficiency
A Calkin, M Lassila, J Forbes, M Cooper, K Jandeleit-Dahm, T Allen
Rosiglitazone is a PPAR gamma agonist currently used for the treatment of type II diabetes and is considered to mediate its effect primarily through controlling insulin sensitivity. Further studies have demonstrated a role for this drug in attenuating atherosclerosis in insulin resistant animals, albeit this has been associated with changes in glucose and insulin levels. We have demonstrated, in a model of insulin deficiency, that rosiglitazone attenuates atherosclerosis by up to sixty percent after 20 weeks of treatment. This was associated with reductions in oxidative stress as measured by chemiluminescence. There was no effect of rosiglitazone on glucose, cholesterol or triglyceride levels. Diabetes was also associated with an increase in albumin excretion which was attenuated by rosiglitazone. These findings demonstrate that rosiglitazone may confer vascular and renal protection in diabetes, independent of its effects on insulin sensitivity and/or glycaemic control. Further studies will identify the pathways by which these effects are mediated.

Grants and other Funding
- AVBS Travel Grant to attend the International Vascular Biology Meeting A Calkin.
- Diabetes Australia Research Trust, The role of coenzyme Q10 and statins in the prevention of diabetes related atherosclerosis. R O'Brien, T Allen.

- Commercially Funded Research Activities. Kao Coporation, Role of DAG oil in diabetic atherosclerosis. P Nestel, T Allen.

- Dairy Australia. Role of CLA in diabetic atherosclerosis. P Nestel, T Allen.

Presentations
- European Association for the Study of Diabetes, Germany. T Allen. Role of pyridoxamine in atherosclerosis.

- International Maillard Symposium, USA, T Allen. Pyridoxamine reduces atherosclerosis in diabetes-associated atherosclerosis.

- International Maillard Symposium, USA, T Allen. Blockade of AGES reduces atherosclerosis in diabetes-associated atherosclerosis.

- 13th International Vascular Biology Meeting; Toronto, Canada – A Calkin, "Rosiglitazone attenuates Atherosclerosis in a Model of Insulin Deficiency'.

- Australian Health & Medical Research Congress; Sydney – A Calkin "Rosiglitazone attenuates atherosclerosis in a model of insulin deficiency'.

- Australian Diabetes Society Annual Meeting; Sydney – A Calkin "PPAR gamma agonists confer vascular protection independent of their effects on metabolism".

ANGIOTENSIN II & DIABETIC COMPLICATIONS LABORATORY

Zemin Cao

Head
Zemin Cao - MBBS MD

Senior Scientific
Jasefa Peta

Professional & Technical Staff
Wendy Huo

Visiting Scientists
Dr ShuHua Han - Changchun, China

Students
Yen Pham - Honours (Monash)

Research Projects

Roles of CDA1 in Atherosclerosis

Yen Pham, Mark Cooper, Ban-Hock Toh, Zemin Cao,and Zhonglin Chai

CDA1 expression in the atherosclerotic plaques of the mouse aorta has been determined by immunohistochemistry staining and quantitative real-time PCR. CDA1 expression is found to be increased in the atherosclerotic aorta comparing to the control animals and the increase attenuated by the angiotensin converting enzyme (ACE) inhibitor, suggesting the potential role of CDA1 in atherosclerosis development by mediating angiotensin action. In vitro studies has been carried out in cultured mouse vascular smooth muscle cells and monocyte/macrophage cell line RAW. The increase of CDA1 expression in the mouse vascular smooth cell line has been found to be correlated with the increases of profibrotic growth factor CTGF and matrix proteins, suggesting CDA1's involvement in the profibrotic response of the vessles to atherosclerosis.

Cloning and Characterization of CDA1 promoter

Shuhua Han, Mark Cooper, Ban-Hock Toh, Zemin Cao,and Zhonglin Chai

The regulation of cell division Autoantigen 1 (CDA1) is studied by making CDA1 promoter driven luciferase reporter constructs. The upstream genomic DNA fragments of the CDA1 gene of approximately 6 kb have been cloned from mouse and human genomes. The construct with luciferase reporter gene driven by the full-length CDA1 upstream fragment and several constructs with shortened fragments have been made. The promoter activity of those constructs have been determined in mouse vascular smooth muscle cells and other cell lines. The regulation of the promoter activity by TGF-b1 and other growth factors is currently under investigation.

CDA1 and Extracellular Matrix Protein Production

Josefa Pete, Mark Cooper, Ban-Hock Toh, Zemin Cao,and Zhonglin Chai

CDA1 is hypothesized to have direct regulation of the profibrotic growth factor CTGF and extracellular matrix proteins (ECMs). Kidney cell lines stably expressing siRNA have been established that knock down endogenous CDA1 level to the 30% of the control cell level. In those CDA1 knocked down cells, CTGF, Collagen 1 and 3 and fibronectin gene expression levels are significantly decreased, suggesting that CDA1 is basally required for the expressing of CTGF and most of the ECMs. Experiments are currently carried out to demonstrate CDA1 would also be required for TGF-b1's stimulation of its target genes including CTGF and ECMs. It is expected to show CDA1 is an important mediator of TGF-b signaling by locating upstream of CTGF.

Molecular Mechanisms of CDA1 actions in cells

Yugang Tu, Greg Rice, Mark Cooper, Ban-Hock Toh, Zemin Cao and Zhonglin Chai

A panel of evidences indicates that CDA1 has multiple biological functions involved in cell proliferation, TGF-b signaling and nucleosome assembly and transcriptional factor activities in neurons. We are interested in understanding the molecular mechanisms as how CDA1 acts as a cell growth inhibitor and mediate the TGF-b signaling. We are taking proteomics approach in collaboration with Greg Rice to look at the effect of CDA1, by either overexpressing or knockdown, on the global protein expression and modifications. We are interested in identifying the proteins interacting with CDA1 by proteomics. Western blotting and Co-immunoprecipitation are also carried out in the same time to identify the proteins that are likely affected by and co-complexed with CDA1.

CDA1 and Cell Proliferation

Yiu-Chee Yuen, Mark Cooper, Ban-Hock Toh, Zemin Cao and Zhonglin Chai

Our published data showed overexpression of CDA1 in HeLa cells arrested cell division that required the phosphorylation of CDA1 by cyclin-dependant kinases. We are now investigating the effect of CDA1 knock down on the cell division. As expected, the preliminary data showed siRNA knock down of CDA1 resulted in faster cell proliferation by cell numbers. The mechanisms of this effect are yet to be studied.

Roles of Glucocorticoids in Cardiomyocyte Hypertrophy and Their Regulation Through 11bHSD1 by Angiotensin II and TGF-b1

Zhonglin Chai, Mark Cooper, Zygmunt Krozowski, and Zemin Cao

In collaboration with Zygmunt Krozowski, we are studying the relationship between Angiotensin II and 11bHSD1 in heart. The preliminary data showed that 11bHSD1 is expressed in neonatal rat cardiofibroblasts and has predominant reductase activity. However, the protein expression of this enzyme is inhibited by TGF-b1, but stimulated by angiotensin II. A strong 11b-hydroxysteroid dehydrogenase activity is detected in neonatal cardiomyocytes suggesting that the mineralocorticoid receptor (MR) is protected by an 11b-dehydrogenase from being activated by the intracellular glucocorticoid. Adenovirus expressing MR, 11bHSD1 and 11bHSD2 have been made and will be used to study the effects of glucocorticoids on cardiomyocyte hypertrophy.

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Research Projects

Advanced glycation end product cross-link breakers in diabetic renal disease

J Forbes, V Thallas, M Thomas, H Haller, M Cooper

This study initially involved preclinical testing of the AGE cross-link breaker ALT-711 (alagebrium) which may provide a new therapy for the treatment of diabetic nephropathy. Intervention with ALT-711 from weeks 16-32 of this study in experimental diabetes had the capacity to not only improve renal functional parameters such as albuminuria but also markers of structural injury including glomerulosclerosis and tubulointerstitial injury. In addition these effects are seen even when combined with traditional RAS blockers. The antifibrotic actions of this agent have stimulated exploration of the role of this compound in various clinical contexts including cardiomyopathy as outlined below. This agent will move into clinical testing in diabetic nephropathy within the next 6 months. Protein kinase C has been suggested as a downstream pathway by which some of these effects are mediated. We have studied the specific isoforms involved and have access to PKC isoform knockouts through collaboration with Prof Haller, Hannover, Germany to further elucidate their involvement in the pathogenic pathways involved in diabetes. Further studies will now be performed to further elucidate its mechanism of action.

Delineation of the effects of AGEs versus glucose on the diabetic heart

G. Soldatos, V Thallas, J Forbes, L Burrell

This study has originally documented that in experimental diabetes there is significant myocardial disease with cardiac AGE accumulation, cross-link formation and increased synthesis of the fibrillar collagen, type III. In experimental diabetes, treatment with ALT-711 inhibited the expression of the AGE receptors, RAGE and AGE-R3 as well as the pro-sclerotic cytokine CTGF. These findings provide evidence for specific receptor and growth factor dependent pathways whereby ALT-711 could attenuate diabetic heart disease in addition to its cross-link breaking actions. These studies have been expanded into the clinical context with patients with diabetes without complications being tested for AGEs as a predictor of progression to cardiac and blood vessel disease. In addition, simplified studies of the differences between damage cause by glucose per se and by AGEs will now be further expanded. Functional studies in the context of diabetes in association with both hypertension or myocardial infarction.

Interactions between the renin-angiotensin system (RAS) and advanced glycation in diabetic nephropathy

J Forbes, M Thomas, V Thallas, Z Cao, M Thomas, H Yamamoto, P Nawroth, M Cooper

Interruption of the RAS with drugs such as ACE inhibitors has proven beneficial in diabetic renal disease. In fact, benefits have been shown beyond the blood pressure effects. In addition in experimental models we have identified synergy between RAS blockade and anti AGE therapies. The findings of this study suggest an interaction between the RAS and advanced glycation in experimental diabetic nephropathy. In particular we have shown evidence that ACE inhibition reduces the accumulation of renal and serum AGEs, possibly via effects on oxidative pathways and protein kinase C. More recently we have discovered that this may be a consequence of the manipulation of splice variants of the receptor for advanced glycation end products (RAGE) which may turn the balance towards the synthesis of a protective form of the RAGE receptor. Kind collaborations with Prof Yamamoto at Kanazawa University, Japan and Prof Peter Nawroth, Heidelberg, Germany will enable the more detailed

investigation of this mechanism by provision RAGE transgenic and knockout mice and vectors etc for cell culture exploration. We have also discovered that exogenous administration of AGEs has the capacity to mimic the changes in the RAS seen in diabetes. Interestingly the converse is also the case with administration of exogenous angiotensin II facilitating the formation of AGEs and upregulating their receptors. These changes are also inhibited by specific blockade of each pathway.

Relative contributions to oxidative stress in diabetes complications from mitochondrial and cytosolic sources

J Forbes, D Thorburn, M. Coughlan, V Thallas, M Brownlee, M Cooper

Oxidative stress is an important contributor to the pathogenesis of diabetic complications. AGEs are both produced by reactive oxygen species (ROS) in addition to producing ROS via their own pathways of damage. This project has already characterised the major contributions to superoxide from within specific organs from both mitochondrial and NADPH oxidase. Mitochondrial respiratory chain dysfunction has been suggested as an acquired defect in diabetes and therefore characterisation of this defect in conjunction with Dr Thorburn from Murdoch Children's Institute has been performed. Prof M Brownlee, Bronx USA is also a collaborator on this project providing specific uncoupling protein and antioxidant knockouts and transgenics. The relative contribution of intracellular vs extracellular glycation has been explored. Administration of a number of antioxidants will be tested as combination therapies and the relative contribution of oxidative stress in the context of advanced glycation end product accumulation will be characterised.

The role of vascular endothelial growth factor and angiopoientins in diabetes induced angiogenesis

B Rizkalla, Z Cao, M Cooper, J Forbes

The present proposal explored the role of newly discovered receptor proteins (Tie-1 and Tie-2) which are believed to be involved in maintaining the integrity of the blood vessel wall. Angiopoietins appear to activate the Tie-2 receptor leading to either inhibition (angiopoietin-1) or sprouting of new vessels (angiopoietin-2). It is suggested that these actions may be of direct relevance to diabetes. For example, in diabetic eyes there is sprouting of new blood vessels which leads to blindness in these patients. A similar event probably takes place in other blood vessel beds including the kidney and heart. These Tie receptors are located on the inner lining of the blood vessel and it is possible they play an important role in diabetic vascular disease.
The project has assessed the changes in these receptors and related proteins in the blood vessel wall within the kidney, heart, eye and mesentery after injury induced by diabetes. This project was completed this year, however the findings will be extrapolated to our other projects.

Tubular dysfunction and filtration of AGE peptides in diabetic renal disease

M Thomas, P Kantharidis, J Forbes, S Thorpe, M Cooper

The renal clearance of organic cations (which includes AGE peptides) is important for the homeostasis of a number of exogenous and endogenous compounds. The organic cation transporters (OCTs) situated on the basolateral surface of renal proximal tubules mediate active cation excretion. Alterations in cation transport may occur in diabetes, although the role of the OCTs has not been previously assessed. These studies have shown reduced gene and protein expression of OCT-1, OCT-2 and OCT-3 in experimental diabetes in association with reduced clearance of the endogenous cation N-methylnicatinamide. Cation clearance was also reduced following AII infusion, independent of transporter expression. Cation clearance and receptor expression were restored following treatment with the ACE inhibitor ramipril, suggesting an additional mechanism to explain treatment-related improvements in creatinine clearance and renoprotection in diabetes following blockade of the renin-angiotensin system. Further extrapolation has shown that AGEs are cleared from the blood via this mechanism and that this process is dependent upon the glomerular filtration rate. The next objective is to identify the source of the AGE peptides which will be performed with the assistance of Prof Thorpe and Baynes, Sth Carolina, USA.

The contribution of AGEs to beta cell secretory defects in diabetes

J. Forbes, R. Slattery, S. Andrikopoulos, V. Thallas, F.Yap, D.Tong

Are ingested AGEs or genetically determined underlying elevations in circulating AGEs responsible at least in part for the initiation of processes which lead to insulin resistance and/or insulin secretory defects. Evidence from rodents infused with AGEs suggests that this is indeed the case. This project involves both rodent studies and samples acquired from patients before the onset of diabetes with known outcomes. This

is probably the most risky project, but at the same time the most novel and may provide some answers for some of those "AGE" old questions! We have currently identified dysfunction and are studying it in more detail by specifically and systematically studying the insulin secretory pathway.

Grants and other Funding

- Juvenile Diabetes Research Foundation, Chief Investigator Program Project Grant , Glycation and Diabetic Nephropathy. J Forbes, L Bach, M Cooper. Jan-Jun 2004

- Juvenile Diabetes Research Foundation, Core Facility in Imaging and Microscopy. J Forbes. Jan-Jun 2004.

- Juvenile Diabetes Research Foundation, Award for the Albert Einstein Centre for Diabetes Complications – Prinicipal Investigator, Michael Brownlee. Chief Investigator project 3 J Forbes, M Thomas, M. Cooper, L. Bach. July 2004-Ongoing.

- National Health and Medical Research Council of Australia, Interactions between advanced glycation and oxidative stress in diabetic renal and cardiac complications. J Forbes, D Thorburn, M Cooper.

- Juvenile Diabetes Research Foundation, Post Doctoral Fellowship. J Forbes.

- National Health and Medical Research Council of Australia, Medical Post Graduate Research Scholarship. The role of advanced glycation in proximal tubular cell dysfunction. M Thomas.

- Australian Kidney Foundation Post Graduate Research Scholarship, Interaction of AGEs, cytokines and adherence molecules in mediating tubulointerstitial and glomerular disease in diabetes: in vitro and in vivo studies. W Burns.

Presentations

- 8th International Symposium on the Maillard Reaction, Charleston, Sth Carolina USA. J. Forbes . Angiotensin Converting Enzyme-1 Inhibition in diabetic nephropathy reduces the accumulation of advanced glycation end products via mediation of soluble RAGE.

- Australian Diabetes Society, Sydney. M. Coughlan. Advanced glycation end products destroy pancreatic islet beta cells via interleukin-1 mediated pathways.

- Australian and New Zealand Society of Nephrology, Adelaide. K Fukami. Angiotensin Converting Enzyme-1 Inhibition in diabetic nephropathy reduces the accumulation of advanced glycation end products via mediation of soluble RAGE.

DIABETES & THE KIDNEY LABORATORY

Karin Jandeleit-Dahm

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Vishal Boolell - AMS (Melbourne)

Research Projects

Effect of HMG CoA reductase inhibition and AT1 receptor antagonism on diabetes accelerated atherosclerosis

Vishal Boolell, Colleen Chew, Markus Lassila, Anna Calkin, Terri Allen, Mark Cooper, Karin Jandeleit-Dahm

In preliminary results we could demonstrate a significant anti-atherosclerotic effect of the HMG CoA reductase inhibitor rosuvastatin and the AT1 receptor blocker candesartan on diabetes associated atherosclerosis. There were dose dependent effects and we will further investigate the effect of combination therapies of rosuvastatin and candesartan in the diabetic apoE knockout mouse, a model of accelerated atherosclerosis. In addition, we are in progress of investigating the pathways involved with the anti-atherosclerotic effects of rosuvastatin and candesartan. Furthermore, there appears to be an important link to advanced glycation end products and we are further investigating this aspect of the study.

Effect of HMG CoA reductase inhibition and AT1 receptor antagonism on diabetes accelerated renal injury

Sara Giunti, Markus Lassila, Anna Calkin, Terri Allen, Mark Cooper, Karin Jandeleit-Dahm

Preliminary results have confirmed renoprotective effects of candesartan and rosuvastatin in the accelerated renal disease seen in diabetic apoE KO mice. These changes are in the context of effects on macrophage infiltration, collagen deposition and growth factor expression. Both interventions also affect advanced glycation pathways and we are currently investigating this in more detail.

Accelerated nephropathy in diabetic apolipoprotein E knockout mouse- role of advanced glycation end products

Markus Lassila, Kwee K Seah, Terri Allen, Vicki Thallas, Merlin Thomas, Riccardo Candido, Wendy C Burns, Josephine Forbes, Anna Calkin, ME Cooper, Karin Jandeleit-Dahm

Advanced glycation end products (AGEs) have been shown to play a pivotal role in the context of diabetes for the development and progression of diabetic nephropathy. In this project we have investigated the role of AGEs in the kidneys of diabetic aopE which show an accelerated form of renal disease similar to the accelerated atherosclerosis in this model. This was associated with increased glomerulosclerosis, tubulointerstitial fibrosis, albuminuria, collagen and growth factor expression. Inhibition of AGE accumulation in the kidney with two different approaches, aminoguanidine and the cross-link breaker Alteon 711, reduced renal structural and functional changes as well as collagen deposition and growth factor expression. This work has been published in the Journal of the American Society of Nephrology in 2004 (2004, 15: 2125-38) and was awarded the Baker Publication Prize for September.

Effect of dual ACE and NEP inhibition on accelerated atherosclerosis and renal injury in diabetic apoE KO mice

Karin Jandeleit-Dahm, Elske Quak, Markus Lassila, Belinda Davis, Louise Burrell, Colin Johnston, ME Cooper

Dual inhibition of ACE and neutral endopeptidase (NEP) has shown superior blood pressure lowering and renoprotective effects in diabetes. In this project we have investigated the effects of omapatrilat on accelerated atherosclerosis and renal injury in diabetic apoE KO mice and have compared these effects with single ACE inhibitor treatment. Omapatrilat had anti-atherosclerostic and renoprotective effects superior to ACE inhibition alone. The manuscript has been submitted to Journal of Hypertension.

Effect of growth factor tyrosine kinase inhibition on atherosclerosis and renal injury in diabetic apoE KO mice

Markus Lassila, ME Cooper, Karin Jandeleit-Dahm

We were able to demonstrate that tyrosine kinase inhibition of the PDGF receptor is able to reduce accelerated atherosclerosis in diabetic apoE KO mice (ATVB 2004, 24: 935-42). This treatment also demonstrates renoprotective effects. We have completed studies showing reductions in albuminuria, glomerulosclerosis index and tubulointerstitial injury with imatinib. These changes were in association with reductions in macrophage infiltration, expression of PDGF and other profibrotic growth factors such as CTGF and TGF-beta. This paper has been accepted for publication in the Journal of the American Society of Nephrology and is published in the February issue.

Role of plasminogen activator inhibitor –I in diabetic nephropathy

Kei Fukami, M Lassila, A Calkin, Kitching, M Cooper, K Jandeleit-Dahm

Studies using plasminogen activator inhibitor-1 (PAI-1) knock-out and transgenic mice aim at finding out whether PAI-1 plays a role in the development in the diabetic nephropathy. A group of both strains as well as of appropriate wild-type mice were made diabetic with streptozotocin or left non-diabetic and the progression of diabetic nephropathy was followed for 24 weeks. We have made some interesting observations between the WT animals and the PAI KO animals. We are currently confirming the genotypes of all animal groups and have started to perform the RNA extractions for gene expression studies including TGF-beta, CTGF and metalloproteinases MMP-2 and MMP-9.

Peroxisome proliferator- activated receptor alpha and gamma in accelerated atherosclerosis and diabetic nephropathy

Anna Calkin & Chris Tikellis, Terri Allen, Mark Cooper

PPAR alpha and gamma agonists confer renoprotective effects in diabetic nephropathy reducing inflammation, growth factor expression and oxidative stress. We are currently investigating in detail effects on reactive oxygen species production and NADPH subunit expression on renal injury.

Clinical associations between advanced glycation end products and cardiovascular complications in diabetic patients

Georgia Soldatos, Richard O'Brien, Bronwyn Kingwell, Mark Cooper, Karin Jandeleit-Dahm

We have recruited more than 50 patients from the Alfred Hospital Diabetes clinics and have performed measurements of vascular compliance, echos diastolic dysfunction and intima media ratio as well as a range of blood and urine tests. We are currently measuring AGEs in the blood of these patients and will then correlate these findings with vascular and cardiac dysfunction as well as compare them to non-diabetic patients with cardiovascular disease and normal subjects.

Grants and other Funding

- NHF Postdoctoral Clinical Fellowship: Karin Jandeleit-Dahm. Associations between advanced glycation pathways and oxidative stress with early cardiovascular disease in diabetic patients.

- NHMRC Project grant: Role of advanced glycation end products in mediating diabetes associated atherosclerosis. Mark Cooper, Karin Jandeleit-Dahm.

- National Heart Foundation Project Grant: The role of advanced glycation end products and related growth factor dependent pathways in diabetes accelerated atherosclerosis. Mark E. Cooper, Karin Jandeleit-Dahm.

- Diabetes Australia Research Trust. Project grant. Cardiac specific overexpression of mitochondrial superoxide dismuatse limits the myocardial complications of type 1 diabetes. Rebecca Ritchie, David Kaye, Karin Jandeleit-Dahm, Grant Drummond

- JDRF Program Grant, Principal Investigator Mark E Cooper. Project 4: The roles of hemodynamic and metabolic factors in mediating diabetes associated atherosclerosis, Chief Investigator Karin Jandeleit-Dahm

Commercially Funded Research Activities

- Astra-Zeneca, Role of HMG-CoA reductase inhibition and AT1 receptor antagonism in diabetes associated renal injury and other vascular complications. M Cooper, K Jandeleit-Dahm.

Presentations

- Avandia and beyond, Specialist weekend (June 2004, Melbourne): K Jandeleit-Dahm: Pleiotropic effects of PPAR agonists in diabetes related atherosclerosis

- Vascular Disease & Diabetes, Baker & George Institute for International Health Symposium, Noosa. K Jandeleit-Dahm (Chair of Vascular complications in diabetes session)

- Australian Health and Medical Research Congress, Sydney November 2004: K Jandeleit-Dahm: Pleiotropic effects of statins and PPAR agonists in atherosclerosis

- HSANZ (Hematology Society of Australia and New Zealand), ANZSBT, ASTH Annual Scientific meeting 2004, Melbourne: K Jandeleit-Dahm: The vascular wall in diabetes

- Maillard Meeting, September 2004, Charleston: late intervention with pyrodoxamine prevents diabetes associated atherosclerosis. D Brasacchio, J Forbes, K Jandeleit-Dahm, M Cooper, T Allen

- Maillard Meeting, September 2004, Charleston: Role of advanced glycation end products in accelerated renal injury in the diabetic apoE KO mouse. K Jandeleit-Dahm, M Lassila, T Allen, M Cooper.

- EASD Annual Meeting Munich, 2004: Role of advanced glycation end products in accelerated renal injury in the diabetic apoE KO mouse. K Jandeleit-Dahm, M Lassila, T Allen, M Cooper.

- EASD Annual Meeting Munich, 2004: Imatinib reduces diabetes associated atherosclerosis in the diabetic apoE KO mouse. M Lassila, ME Cooper, T Allen, K Jandeleit-Dahm

GENES AND DIABETIC COMPLICATIONS LABORATORY

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Research Projects
Diabetes is by far the most common metabolic disorder that is associated with significant morbidity and mortality. The key to better understand this complex disease is to identify the genes and their products, be they novel or known, which contribute to or confer protection against the disease, as well as the effect of diabetes on the transcriptional regulation of genes.

Recent advances in chromatin biology have enabled us to design experiments to better understand the process of gene transcription and how diabetes impacts on the transcription of key genes in diabetic complications. Also, the establishment of a new proteomics laboratory at the Baker has enabled us to start looking at post-translational modification of proteins that are important in diabetes but also regulating gene transcription.

Mechanistic studies in AGE induced renal epithelial to myofibroblast transition
W Burns, P Kantharidis
One of the important mediators diabetic nephropathy appears to be the epithelial-to-mesenchymal transition (EMT) of tubular cells into a fibrogenic myofibroblast phenotype. These changes lead to the accumulation of extracellular matrix (ECM) components and result in progressive renal scarring. This study examines the role of advanced glycation end products (AGE) and underlying mechanisms in the induction of EMT in diabetes. The exposure of a number of cell types, including rat proximal tubule cells (NRK52-E) to AGE-BSA results in EMT, with loss of e-cadherin expression, de novo expression of aSMA and the induction of a myofibroblastic phenotype. Increased expression of ECM proteins (collagen IV and fibronectin) and the fibrogenic growth factors connective tissue growth factor (CTGF) was also observed. AGEs are a potent stimulus for EMT. Some of this effect appears to be mediated via CTGF, which independently induces EMT as demonstrated in our adenoviral studies. In more recent studies we have identified a family of transcription factors that mediate the transcriptional repression events observed in EMT. These factors are also involved in transition of breast and other cancer cells to a more metastatic phenotype. In preliminary experiments AGEs, TGFb and CTGF appear to activate these factors in NRK52-E cells. The pathways involved in the activation of the transcriptional repressors are currently being investigated, as are the genes that targets for repression and activation. These studies provide important insights into the mechanism of progressive fibrogenesis in diabetes.

Gene profiling analysis of RNA from aorta control, diabetic and high fat-fed ApoE knockout mice
A Calkin, T Allen, K Jandeleit-Dahm, P Kantharidis
The focus of this project is the identification of genes and proteins that are associated with the development of atherosclerosis in the ApoE mouse model of diabetes. In particular, the current studies aim to determine whether atherosclerosis in the diabetic model is different to that found in the high fat fed model, or a simply a more accelerated form of the same disease. Animal experiments have been performed and RNA isolated from the mouse aorta. The small amount of RNA obtained from each aorta has proven to be a challenge however the array experiments have been conducted successfully and the data is continuing. The expression of a number of genes in the aortas of diabetic animals is altered when compared to control animals.

IGF-BP6 structural determination
L Bach (Uni Melb, A&RMC), R Norton (WEHI), P Kantharidis
Insulin like growth factors (IGFs) are important mediators of growth. IGF binding proteins (IGFBPs) 1-6 regulate IGF actions and have IGF-independent actions. The C-terminal domains of IGFBPs contribute to high-affinity IGF binding and modulation of IGF actions and confer some IGF-independent properties, but understanding how they achieve this has been constrained by the lack of a three-dimensional structure. The solution structure of the C-domain of IGFBP-6 was determined using nuclear magnetic resonance (NMR).

The domain consists of a thyroglobulin type 1 fold comprising an alpha-helix followed by a loop, a three-stranded antiparallel beta-sheet incorporating a second loop, and finally a disulfide-bonded flexible third loop. The IGF-II binding site on the C-domain was identified by examining NMR spectral changes upon complex formation. It consists of a largely hydrophobic surface patch involving the alpha-helix, the first beta-strand, and the first and second loops. The site was confirmed by mutagenesis of several residues, which resulted in decreased IGF binding affinity. The IGF-II binding site lies adjacent to surfaces likely to be involved in glycosaminoglycan binding of IGFBPs, which might explain their decreased IGF affinity when bound to glycosaminoglycans, and nuclear localization. This structural information provides a framework for understanding the roles of IGFBP C-domains in modulating IGF actions and conferring IGF-independent actions, as well as ultimately for the development of therapeutic IGF inhibitors for diseases including cancer. This work has now been published.

Molecular biology of ACE2

C Tikellis, P Kantharidis, M Cooper

The renin-angiotensin system plays a pivotal role in mediating a number of diabetic vascular complications. Using molecular and peptide biological approaches in collaboration with the Peptide Biology group we are exploring expression and the role of ACE2 in the diabetic kidney, heart and retina.

Epigenetics and diabetes

P Kantharidis

The direct impact of diabetic disease on gene transcription is still poorly understood. Many changes in gene expression have been reported, the significance of which remains to be determined. Since many proteins are modified by advanced glycation in diabetic disease, we have postulated that chromatin function may be altered or even impaired and hence effects on gene expression. This project focuses on identifying the modification of transcription factors and other chromatin elements using the proteomics facility at the Baker. Subsequent experiments will determine the functional significance of such modifications.

CELL BIOLOGY LABORATORY

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Michael Ditiatkovski - PhD Student, (Monash)

Research Projects

Functional Blockade of Fibroblast Growth Factor Receptors Prevents Smooth Muscle Cell Accumulation in Atherosclerotic Lesions of Apolipopprotein E-Deficient Mice

T Raj, P Kanellakis, G Jennings, A Bobik A, Agrotis

The vascular smooth muscle cell is the central cell component involved in the fibroproliferative response in atherosclerosis. As lesions progress in complexity, smooth muscle cells migrate into the subendothelial space and proliferate, contributing to lesion growth. Fibroblast growth factors are known to potently stimulate vascular smooth muscle cell proliferation and whilst they are known to be expressed in lesions, together with their receptors, their role in atherogenesis is not known. We examined their role in the development of atherosclerotic lesions using a specific inhibitor of fibroblast growth factor signaling SU5402. Initially we examined the effects of SU5402 on development of the intima in ApoE-/- mice. Two weeks after ligating the carotid artery of control mice a large occlusive intima developed consisting of smooth muscle cells and macrophages. Treatment with SU5402 reduced intima size by nearly 80%. Macrophage accumulation in the intima was also reduced, by nearly 70%. In ApoE-/- mice fed a high fat diet for 8 weeks SU5402 reduced atherosclerotic lesion size by 50%. Macrophage numbers in the lesions were also reduced, by 50% and vascular smooth muscle cell numbers by nearly 80%. We conclude that fibroblast growth factor receptors play major roles in intimal smooth muscle cell proliferation, smooth muscle cell accumulation within atherosclerotic lesions, and the development of atherosclerotic lesions. Tyrosine kinase inhibition of fibroblast growth factor receptors appears to be a novel therapeutic option to retard the development of atherosclerosis.

NKT Cells and Development of Atherosclerosis

K To, B-H Toh, A. Bobik

It is well known that activated T cells and professional antigen presenting cells infiltrate atherosclerotic lesions. In lesions lipid antigens can be presented to T cells as part of a complex with the CD1d-molecule, which is displayed on certain antigen presenting cells. Recently CD1d-restricited NKT cells have been identified in human lesions but their significance for the development of atherosclerosis is not known. We investigated their role in the development of atherosclerosis in ApoE-/- mice fed a high fat diet. Since NKT cells, defined by the expression of the NK1.1 receptor, emigrate from the thymus at days 5-7 after birth, we removed these cells from ApoE-/- mice by subjecting them to thymectomy at day 3 after birth. Control mice underwent sham thymectomy. When the mice reached 6-weeks of age they were fed a high fat diet for the ensuing 8 weeks. Lesion size in the neonatally thymectomized mice was on average reduced by nearly 60%. Macrophage accumulating in the lesions was also reduced by a similar amount where as the T-cell population was reduced by nearly 75%. This effect on lesions was not a consequence of any reduction in plasma cholesterol levels which were on average elevated by nearly 3-fold in mice fed the high fat diet. RT-PCR analyses of mRNA from the lesions indicated an absence of the semiinvariant T cell receptor Va14-Ja281 a-chain confirming the depletion of CD1d-restricted NKT cells. Foxp3 mRNA, a marker of CD4+CD25+ T cells was also absent from the lesions. In contrast to these effects of neonatal thymectomy on lesion development, subjecting mice to thymectomy when the reached 6-weeks of age had no effect on lesion development. We conclude that CD1d-restricted NKT cells contribute to the development of atherosclerosis in ApoE-/- mice and very likely also in humans.

CD4+ T Cells Do Not Influence Development of Atherosclerotic Lesions in Apolipopprotein E-Deficient Mice

K To, B-H Toh, A Bobik

Activated T lymphocytes are found in substantial numbers in human atherosclerotic lesions, both in the very early stages of development and in advanced plaques. Despite their presence, their potential involvement in lesion development and progression is still controversial. For example, ApoE-/- crossed to Rag2-/- mice exhibit reductions in lesions only when fed a normal diet. In contrast, in ApoE-/- mice deficient in CD4 T cells lesion size at the aortic sinus is not decreased whilst in the thoracic and abdominal aorta lesions increase. To further explore the role of CD4 T cells we

depleted this population by administering anti-CD4 antibodies, commencing 3 weeks before placement of the mice on a high fat diet and for the subsequent 10-weeks whilst on the high fat diet. The treatment completely depleted CD4+ T cells from lymph nodes without affecting either the CD8+ T cell population or B-lymphocytes. CD4+ T lymphocytes were also reduced in atherosclerotic lesions by more than 80%. Despite these large reductions in CD4+ T cells, lesion size was unaffected as was macrophage accumulation in the lesions. Our results suggest that CD4+ T lymphocytes do not contribute to the development of atherosclerotic lesions in ApoE-/- mice. However, they may influence the type of lesion that develops, possibly promoting the development of unstable fibrofatty lesions.

G-CSF-primed Hematopoietic Stem Cells Accelerate Recovery and Improve Function after Myocardial Infarction, Predominantly by Promoting Endogenous Repair Programs

P Kanellakis, N Slater, D Curtis, S Jane, A Bobik

The heart has a very limited regenerative capacity to heal following infarction. Attempts to heal the infarcted heart have included the isolation of specific populations of bone marrow derived cells, including stem cells that can apparently transdifferentiate into cardiomyocytes and/or endothelial cells and smooth muscle cells. We have shown that administration of stem cell factor together with granulocyte-colony stimulating factor to mobilize bone marrow progenitor cells can improve cardiac function after myocardial infarction as well as improve healing of the infarcted region. We have now extended this study to determine the extent to which bone marrow derived progenitor cells contribute to the cardiomyocyte, endothelial and vascular smooth muscle cell populations in infarcted regions. Bone marrow derived cells were engineered to express DS RED using a retrovirus and then injected into lethally irradiated mice. After 4 weeks the mice were subjected to transient coronary artery ligation for one hour, then treated with either stem cell factor plus granulocyte colony stimulating factor or vehicle and the infarcted regions examined one and four weeks later. The cytokine treatment greatly increased the number of DS RED cells that accumulated in the infarcted region, both one and four weeks after infarction. At these times over 80% of the DS RED expressing cells also expressed CD45. Substantial numbers of DS RED cells were also found to express CD68, a macrophage marker. However, we could not detect DS RED cells expressing either alpha-actinin, alpha smooth muscle cell actin or von Willebrand factor. Our results suggest that transdifferentiation of cytokine mobilised bone marrow derived stem cells into either cardiomyocytes, endothelial cells or vascular smooth muscle cells is a very rare event and minor contributor to healing of the infarcted heart. The increase in immature cardiomyocytes and vessel density we have observed following treatment with stem cell factor and granulocyte-colony stimulating factor appear due to the cytokines promoting endogenous repair programs, probably stimulating indirectly the proliferation of cardiac derived stem cells and local endothelial and vascular smooth muscle cells to improve heart function.

Effects of Cytokines on Cardiac Function After Myocardial Infarction

P Kanellakis Slater, D Curtis, S Jane, A Bobik

Preserving left ventricular function and preventing adverse left ventricular remodeling after myocardial infarction is a key goal of current cell-based and cytokine therapies. We compared the efficacies of three cytokine therapies on their ability to improve cardiac function after myocardial infarction in mice. Treatment with a combination of stem cell factor plus granulocyte factor improved LV developed pressure and LV +dp/dt as did treatment with granulocyte-colony stimulating factor alone. Improvement was highly dependent on the time therapy was commenced and was most effective when commenced 2 hours after the infarction. The most effective combination of cytokines tested was a combination of granulocyte-colony stimulating factor and erythropoietin. This therapy normalized the depressed LV developed pressure and LV +dp/dt observed one month after myocardial infarction. Current studies are aimed at investigating the mechanism by which this combination of cytokines improves cardiac function.

Granulocyte macrophage-colony stimulating factor and atherosclerosis in ApoE-/- mice

M Ditiatkovski, P. Kanellakis, B-H Toh, A. Bobik

Granulocyte macrophage-colony stimulating factor (GM-CSF) is upregulated in human atherosclerotic lesions, suggesting potential roles for this cytokine in the development/progression of atherosclerosis. GM-CSF is known to stimulate macrophage proliferation, superoxide anion production by macrophages as well as the expression of myeloperoxidase. We found that GM-CSF expressing cells in atherosclerotic lesions of ApoE-/- are increased during the early development of atherosclerosis. To determine the potential role of this cytokine in lesion development we generated a GM-CSF deficient ApoE-/- mouse (GM-CSF-/-ApoE-/-). Feeding 6-week old GM-CSF-/-ApoE-/- mice a high fat diet for 8 weeks increased blood cholesterol levels 3-4 fold, levels similar to those achieved in ApoE-/- mice fed a high fat diet. However, contrary to expectations GM-CSF-/-ApoE-/- mice developed more severe lesions as indicated by Oil Red O staining; on average lesions were double the size of those observed in ApoE-/- mice. There was also nearly double the number of macrophages in lesions of GM-CSF-/-ApoE-/- mice compared to ApoE-/-. Whilst GM-CSF is normally considered to

be an inflammatory cytokine and therefore might be expected to promote lesion development, its presence in atherosclerotic lesions of ApoE-/- mice retards lesion development. The mechanisms by which GM-CSF retards lesion development are currently under investigation, particularly its ability to influence apoptosis and angiogenesis.

TRANSCRIPTIONAL MECHANISMS REGULATING THE EXPRESSION OF FIBROBLAST GROWTH FACTORS AND RECEPTORS IN MOUSE AORTIC SMOOTH MUSCLE CELLS AND MACROPHAGES

T Raj, R Dilley, A Bobik, A Agrotis

Atherosclerosis and its associated coronary and cerebral complications continues to represent a major public health problem. Vascular smooth muscle cells (VSMCs) and macrophages constitute two of the main cell types involved in the development of atherosclerotic lesions, but the growth factor signalling mechanisms that underlie such involvement remain to be elucidated. Here, we characterized the expression profile of the 22 ligands of the fibroblast growth factor (FGF) superfamily and their four high affinity signalling receptors (FGFRs1-4) in mouse aortic VSMCs and RAW 264.7 macrophages. Under normal proliferative conditions, the VSMCs expressed FGF-1, FGF-2, FGF-8, FGF-11 and FGF-18, and FGFR-1, FGFR-2 and FGFR-3. In contrast macrophages expressed only FGF-11, and no FGFRs. However, under culture conditions in which promoter CpG methylation was inhibited either alone (exposure to 5-aza-2'-deoxycytidine), or combined with inhibition of histone deacetylation (exposure to trichostatin A) levels of FGF ligands together with FGFRs were dramatically increased in both cell types, indicating that transcriptional repression was a major mechanism regulating FGF/FGFR genes and involved DNA methylation- and histone acetylation-dependent mechanisms. Furthermore, exposure of the two cell types to inflammatory mediators, including low-density lipoproteins, lipopolysaccharide, and the cytokine CD-40L, could abrogate this transcriptional repression of FGF/FGFRs genes. Our data are the first to define mechanisms that could regulate FGF/FGFR transcription in VSMCs and macrophages within atherosclerotic lesions.

ASSOCIATION OF TGF-b GENE POLYMORPHISMS AND VENTRICULAR HYPERTROPHY IN HYPERTENSIVE INDIVIDUALS

A Agrotis, G Kostolias, D Kaye, A Bobik

Evidence is emerging as to the critical role that polymorphism within growth factor genes could play in the pathophysiology of the cardiovascular system. In particular, transforming growth factor-b (TGF-b) is a multifunctional cytokine secreted by several cell types, including peripheral blood mononuclear cells, endothelial cells and vascular smooth muscle cells. As a potent influencer of extracellular matrix production and cellular growth, perturbations of TGF-_1 levels have been implicated in fibrogenesis, hypertension, and ventricular hypertrophy. Significantly, polymorphisms have been identified within the pro-peptide region of TGF-b1 which have been linked to its altered expression, and which have been associated with hypertension and fibrosis. With this information, we have examined whether specific TGF- b1 polymorphisms are associated with ventricular hypertrophy amongst a cohort of hypertensive individuals in the Melbourne population. DNA from forty four patients were subjected to PCR for 500 bp region encompassing the pro-peptide region of TGF-b1 in which two previously identified single nucleotide polymorphisms (SNPs) have been identified, a T‡C substitution at codon 10 (replacing the amino acid leucine with proline) and a G‡C substitution at codon 25 (replacing arginine with proline). Screening for the existence of polymorphism at each site was carried out with diagnostic restriction endonuclease digestion, and electrophoresis of fragments in 7% acrylamide gels. Interestingly, our preliminary data show that polymorphism at codon 10 is associated with specific parameters of left ventricular hypertrophy, possibly linked to alterations in the level of circulating TGF- b1 in such individuals. We are now screening a larger cohort of hypertensive individuals to confirm this association.

Grants and Funding

- NHMRC Program Grant, Advanced Heart Disease and Heart Failure. G Jennings, A Bobik, A Dart, M Esler, D Kaye

Commercially Funded Research Activities

- Amgen Inc., Cytokine mobilization of bone marrow derived stem cells for cardiac reparation after infarction. D Curtis, S Jane, A Bobik

Presentations

- XIIIth International Vascular Biology Meeting, Toronto, Canada- A Bobik. The DNA-Binding protein and inflammatory mediator HMGB1 is highly expressed in human aortic fibrofatty lesions.

- Australian Vascular Biology Meeting, Barossa Valley, SA-T Raj. Transcriptional mechanisms regulating the expression of fibroblast growth factors and receptors in mouse aortic smooth muscle cells and macrophages.

- Australian Health & Medical Research Congress, Sydney, NSW-A Bobik.HMGB1 and Atherosclerosis.

CLINICAL PHYSIOLOGY LABORATORY

Bronwyn Kingwell

Head
Bronwyn Kingwell - BSc (Hons), PhD

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Ying Fu - MSc (0.5 EFT)

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Anthony White - MBBS, PhD (Monash)
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Research Projects
Large artery stiffness as a risk marker and therapeutic target; structural and genetic aspects
Large artery stiffness is a newly identified independent risk marker for cardiovascular disease. Through an integrated series of human, animal and cell culture studies we are investigating the mechanisms by which large artery stiffness increases cardiovascular risk.

These studies are directed towards
- improving cardiovascular risk prediction
- reducing risk through therapeutic targeting of large artery stiffness

Hypotheses
- That identification of important structural and genetic determinants of large artery stiffness will aid in cardiovascular risk prediction.
- That unfavorable haemodynamics caused by large artery stiffening promote myocardial ischaemia.
- That large artery stiffness can be improved by increasing the elastin/collagen ratio in the walls of arteries through ACE inhibition.

Background
Large artery stiffening causes pulse pressure elevation and both parameters are related to cardiovascular mortality independently of other major risk factors. The mechanism underlying this relationship could be due to similar disease progression in the coronaries and aorta. Alternatively, intrinsically stiff large vessels may promote atherosclerosis throughout the circulation through pulse pressure elevation and subsequent mechanical fatigue and endothelial disruption. In addition, elevated aortic stiffness and pulse pressure impair coronary perfusion and increase myocardial work. Understanding the factors contributing to large artery stiffening is thus likely to aid risk prediction and identify individuals particularly responsive to therapy directed at the large arteries. Patients with ischaemic heart disease are likely to derive particular benefit from such therapies. Many factors influence large artery stiffness but age, atherosclerosis and gender are amongst the most important. The processes underlying these influences ultimately relate to matrix composition which is largely regulated by matrix metalloproteinases (MMPs). Studies in 2004 were directed to understanding how various agents influence matrix composition and thus large artery stiffness.

Pharmacological therapies
We have shown that angiotensin converting enzymes inhibitors reduce large artery stiffness through increasing the elastin to collagen ratio in the artery wall. They do this through modulating expression of both matrix proteins and MMPs. Pharmacological interventions targeted to reduce large artery stiffness are likely to have benefits additional to conventional therapies which may be particularly relevant to ischaemic heart disease.

Gender
Elderly women have stiffer large arteries than men, accounting in part for the greater incidence of isolated systolic hypertension in older women. We have recently linked the more pronounced age-related increase in large artery stiffness in females to hormonal changes and the menopause. In 2004 we conducted cell culture studies which clearly show that female sex steroids promote elastogenic matrix deposition while testosterone deposition of less distensible collagen. In addition to hormonal influences, females have intrinsically stiffer large arteries compared with males. In a study of local school children we have shown that pre-pubertal girls have stiffer large vessels compared with boys but that this difference is no longer evident after puberty. These findings are of particular relevance to the greater incidence of isolated systolic hypertension in women.

Outcomes and Significance
Our work has identified specific structural and genetic determinants of large artery stiffness in relation to cardiovascular risk prediction. We have identified large artery stiffness as an important ischaemic mechanism and have shown that ACE inhibition is an appropriate therapy to improve symptoms and reduce large artery stiffness in patients with peripheral arterial disease. Together these studies may permit more appropriate targeting of therapy in relation to age, CAD severity, gender and genetic background.

Coronary plaque stability
The acute coronary syndromes comprise unstable angina, myocardial infarction and sudden death. An acute coronary syndrome is often the first manifestation of coronary artery disease in a previously healthy individual. In the majority of cases, the cause of acute coronary syndromes is physical disruption of an atherosclerotic plaque in a coronary

artery. Our research seeks to identify markers of such "vulnerable" atherosclerotic plaque which could be used diagnostically. We are assessing biomechanical markers measured using intravascular ultrasound and blood markers measured in samples taken from within the coronary arteries. The blood samples are also being used to study genetic determinants of unstable coronary syndromes. Our studies this year have identified promising markers in all these categories.

Significance: These studies will develop our understanding of the mechanisms contributing to coronary plaque instability and to identify potential plasma, genetic or imaging markers. Recognition of such mechanisms may contribute toward the eventual aim of prospective identification of plaques with the highest probability of spontaneous rupture. (Currently the only way to identify such plaques is retrospectively, after they've caused a heart attack!)

Contraction mediated glucose uptake as a therapeutic target in type 2 diabetes
Type 2 diabetes accounts for over 85% of diabetic patients. The incidence is steadily increasing in Western countries, currently affecting approximately 7.6% of adult Australians and posing a significant health problem in terms of both individual suffering and economic burden. Our recent studies have shown that nitric oxide (NO) is an important mediator of glucose uptake during exercise. This work could contribute to novel approaches to improved diabetic control and prevention of diabetic complications.

Hypothesis
That signaling molecules implicated in the contraction mediated glucose uptake pathway including NO may represent new therapeutic avenues to improve resting glycaemic control in patients with type 2 diabetes.

Background
The UK Prospective Diabetes Study provides evidence that glucose control decreases the risk of diabetes-related end points and has focussed interest on the mechanisms controlling skeletal muscle glucose uptake. Both insulin and muscle contraction increase skeletal muscle glucose uptake through translocation of the GLUT-4 glucose transporter from the cytosol to the plasma membrane. Under physiological conditions, both stimuli are important synergistic mediators of glucose uptake during exercise such that for any given level of contractile activity, insulin further increases glucose uptake.

The signalling pathways activated by insulin and contraction however differ. Patients with type 2 diabetes and insulin-resistant obese Zucker rats have impaired insulin stimulated GLUT-4 translocation, however exercise-stimulated GLUT-4 translocation is normal. Furthermore, despite deficits in insulin mediated GLUT-4 translocation, skeletal muscle glucose utilization during exercise is normal or supranormal in patients with type 2 diabetes. These data therefore suggest that type 2 diabetic subjects may be more reliant on contraction mediated glucose uptake during exercise which partly explains the known beneficial effects of exercise training with respect to glycaemic control. Given the functionality of the contraction pathway in diabetic subjects, signaling molecules activated by contraction might represent an exciting new therapeutic avenue for stimulation of glucose uptake at rest.

NO mediated glucose uptake
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%. This study has recently been extended into type 2 diabetic patients. We have shown that NO dependent mechanisms account for 78% of glucose uptake during exercise in patients with type 2 diabetes and 34% in age, weight and fitness matched healthy individuals. The effect of NOS inhibition was significantly greater in the diabetic subjects compared with controls. Importantly, NOS inhibition had no effect on leg blood flow, arterial blood pressure, insulin or glucose concentrations during exercise. We have interpreted the data to suggest that NO mediated glucose uptake may compensate for impaired insulin action and account for the normal glucose uptake in type 2 diabetic subjects during exercise. This finding suggests that it may be possible to stimulate the contraction mediated glucose uptake pathway at rest by NO donors. We have exciting preliminary data showing that femoral arterial infusion of the NO donor sodium nitroprusside in patients with type 2 diabetes increases glucose uptake compared to the NO-independent vasodilator verapamil. Clinical trials assessing the efficacy of chronic NO donor drugs to improve glucose disposal will proceed in 2005.

Other current studies based on NO mediated glucose uptake involve levels of the 'good' cholesterol, high density lipoprotein (HDL). HDL is inherently lower in patients suffering from Type 2 diabetes and this correlation may be linked to disease progression. Studies in our lab have shown that HDL can increase NO release in the endothelium via direct mechanisms. Activation of NO appears to involve an important enzyme (AMP-activated protein kinase) in glucose and fat metabolism. We placed a provisional Patent on these findings in April and they were subsequently published in the prestigious journal, Proceedings of the National Academy of Sciences (USA) in May. Current studies are examining the links between HDL levels, NO release and glucose uptake in skeletal muscle. If proven, therapies which target this pathway (eg HDL fragments) may have efficacy with respect to reducing the metabolic abnormalities. Such therapies would be particularly relevant to obese patients with blood cholesterol abnormalities or type 2 diabetes who are at significantly greater risk than non-diabetics. Given the increasing prevalence of these conditions to epidemic proportions in

Western countries, the development of new therapeutic targets may significantly reduce morbidity and mortality.

Outcomes and Significance
Over 600,000 Australians have type 2 diabetes and it is estimated that this number will increase substantially to 10% of the adult population over the next 10 years. This work suggests that NO might represent the basis of a novel therapeutic approach to controlling blood glucose. This work could contribute to improved metabolic control and prevention of diabetic complications through new hypoglycaemic agents.

Grants and other Funding
- NHMRC program, Heart failure and its antecedents. G Jennings, D Kaye, M Esler, A Bobik, A Dart, B Kingwell, J Chin-Dusting, X-J Du, J Power

- NHMRC CCRE, Human cardiovascular research influencing clinical practice and community outcomes. G Jennings, A Dart, M Esler, D Kaye, C Reid, J Chin-Dusting

- NHMRC project, A novel mechanism for manipulation of peripheral glucose disposal in patients with Type 2 diabetes. B Kingwell, S Duffy, G McConell.

- NMRC Singapore, The Structural and genetic basis of large artery stiffening in cardiovascular and cerebrovascular disease; risk prediction and therapeutic targeting. B Kingwell, J Cameron, W Cheong.

Presentations
Chair:
1. 2nd Australian Health and Medical Research Congress, Sydney, Australia. BA Kingwell, NHMRC: How do we get funded?

Invited presentations:
1. 20th Scientific meeting of the International Society of Hypertension, Sao Paulo, Brazil. BA Kingwell. Nitric oxide as a metabolic regulator during exercise.

2. 2nd Australian Health and Medical Research Congress, Sydney, Australia. Obesity and Cardiovascular Disease session. BA Kingwell, Inflammation & cardiovascular disease. Is obesity the missing link? (invited plenary).

Abstract presentations
1. Neural, Hormonal and Renal Interactions in Long-term Blood pressure Control. Samode, Rajasthan, India. BA Kingwell. Do central arterial waveforms predict future cardiovascular events better than brachial blood pressures?

2. 77th Scientific Sessions of the American Heart Association. New Orleans, U.S.A. BA Kingwell. Sex steroids modulate expression of matrix proteins and matrix metalloproteinases and may contribute to gender differences in large artery stiffness.

3. 20th meeting of the International Society of Hypertension, Sao Paulo, Brazil. BA Kingwell, Diet but not aerobic exercise training reduces skeletal muscle TNF-a in overweight humans.

4. 20th meeting of the International Society of Hypertension, Sao Paulo, Brazil. BA Kingwell. Cholesterol and large artery stiffness in a hypertensive population.

5. 2nd Australian Health and Medical Research Congress, Sydney, Australia. BA Kingwell. Sex steroids modulate expression of matrix proteins and matrix metalloproteinases and may contribute to gender differences in large artery stiffness.

6. 52nd Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand. BA Kingwell, High Density Lipoprotein and Apolipoprotein AI increase eNOS activity via direct protein association and multisite phosphorylation changes.

7. 52nd Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand. BA Kingwell, The nitric oxide donor, sodium nitroprusside increases glucose uptake across the leg in patients with type 2 diabetes mellitus.

8. 52nd Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand. A. White, A translesional concentration gradient of matrix metalloproteinase-2 (gelatinase A) but not metalloproteinase-3.

9. 2nd Australian Health and Medical Research Congress, Sydney, Australia. AA Ahimastos, ACE inhibition improves clinical walking distance in patients with peripheral arterial disease.

10. 2nd Australian Health and Medical Research Congress, Sydney, Australia. D Henstridge, The nitric oxide donor, sodium nitroprusside increases glucose uptake across the leg in patients with type 2 diabetes mellitus.

CELL BIOLOGY OF DIABETES LABORATORY

Peter J Little

Head
Peter J Little - B Pharm, M Sc, Ph D, ASIA

Professional & Technical
Narin Osman - B. Sc. (Hons) Ph. D.
Julie Nigro - B. Appl. Sci., B.Sc. (Hons) Ph. D. (from July 1st 2004)
Karen Frontanilla - B Appl Sci (Pharm Sci 3rd year)

Visiting Scientists
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Students
Mandy Ballinger - PhD (Monash)
Melanie Ivey - Ph D (Monash)
Stephanie De Dios - PhD (Monash) Graduated Dec. 2004
Julie Nigro - PhD (Monash) Graduated Dec 2004 (until June 30th, 2004)
Soniya Survase - Honours (Monash)
Yifat Biran - RMIT Pharm. Sciences

Research Projects

Regulation of glycosaminoglycan synthesis on vascular proteoglycans by peptide agonists at 7 transmembrane G protein coupled receptors

M Ivey, N Osman, K Frontanilla, W Thomas, P Little

The retention of lipoproteins by proteoglycans in the vascular wall is hypothesized to be the initiating step in atherogenesis. Vasoactive factors are able to influence this phenomenon by stimulating vascular smooth muscle cells to synthesize proteoglycans with longer glycosaminoglycan chains on their core proteins, hence increasing the capacity of the proteoglycans to trap LDL. Endothelin, thrombin and angiotensin II are vasoactive hormones which stimulate 7 transmembrane G protein coupled receptors. We investigated the effects of these agents on proteoglycan synthesis, size and capacity to bind LDL. The signaling pathway through which they stimulate proteoglycan synthesis was also studied. Endothelin-1 stimulates the synthesis of proteoglycans that have longer glycosaminoglycan chains that had a higher capacity to bind LDL. Inhibition of protein kinase C caused a reduction in the size of the proteoglycans, when cells were stimulated with endothelin-1, but inhibition of EGF receptor transactivation had no effect on endothelin-1 stimulated increases in proteoglycan size. Thrombin had a similar effect although its was only partially antagonised by an EGF receptor blocker. We conclude that endothelin-1 and thrombin stimulate important atherogenic changes in proteoglycans but by distinct signaling pathways. This project is ongoing.

Fenofibrate modifies human vascular smooth muscle proteoglycans and reduces LDL binding

J Nigro, M Ballinger, R Dilley, G Jennings, P Little, with T Wight (Hope Heart Institute, Seattle, USA)

Fenofibrate is used to treat elevated plasma triglycerides a condition that is prominent in people with diabetes. The aim is to prevent vascular disease. We have examined the direct effects of fenofibrate that might contribute to its ability to prevent vascular disease. We have examined the direct actions of fenofibrate on human vascular smooth muscle cells (VSMCs) and specifically investigated proteoglycan synthesis and binding to LDL. Fenofibrate inhibited the system that lengthens the glycosaminoglycan (GAG) (carbohydrate) chains on the proteoglycans but it does not change the sulphation pattern or the carbohydrate composition. We used a new technique and instrument, Fluorophore Assisted Carbohydrate Electrophoresis (FACE) to demonstrate that the effect of fenofibrate was on the size and not the composition of the carbohydrate chains. Thus, fenofibrate modifies the structure of vascular proteoglycans by reducing the length of the GAG chains resulting in reduced binding to human LDL, a mechanism which may lead to a reduction of atherosclerosis and cardiovascular disease in people with diabetes treated with fenofibrate. This project was completed and published in 2004.

Regulation of glycosaminoglycan synthesis by inhibitors of protein tyrosine kinases

M Ballinger, J Nigro, A Dart, P Little with A Wilks, S Su (Cytopia Ltd)

Proteoglycans are a family of vascular matrix molecules. The group form part of the extracellular matrix and consist of an individual core protein covalently linked with one or more glycosaminoglycan (GAG) chains. These macromolecules can interact with other vascular entities such as lipoproteins and can also modify the migration and proliferation of vascular cells, events identified as atherogenic. Glycosaminoglycan length influences lipoprotein binding such that longer GAG chains are 'stickier' and retain more lipoprotein particles. The mechanism(s) by which GAG chains are lengthened are currently unclear. The role of the tyrosine

kinases c-Abl, c-Kit and PDGF are being investigated, with the use of specific inhibitors, to elucidate pathways for reducing GAG length, investigate the effects on GAG structure and the resultant lipoprotein binding. This project is assisted by Cytopia Ltd. a biotech company in the Baker building which provides new chemical entities and kinase assays. We have one patent and another in partnership with Cytopia under development. Hopes for a commercial outcome for this project are high.

Regulation of human vascular smooth muscle proteoglycan biosynthesis by biguanides, sulfonylureas and glitazones

P Little, S de Dios , K Frontanilla, E Cawson, M Ballinger, J Nigro, M Ivey

Diabetes is associated with high levels of ischaemic vascular disease leading to strokes, heart attacks, amputations and impotency. Therapy aims to reduce hyperglycaemia with the objective of reducing vascular disease. We are investigating the actions of anti-diabetes drugs in an in vitro model of atherogenesis with a view to understanding and characterising their anti-atherogenic potential. The model is based on the "response to retention" hypothesis and thus the binding of atherogenic lipoproteins to vascular smooth muscle derived proteoglycans (PG). We investigated six new and older generation sulphonylureas and none had any effect on proteoglycan metabolism. Metformin and especially phenformin inhibited proteoglycan biosynthesis but this was due to an inhibition of core protein biosynthesis and not to an effect on glycosaminoglycan synthesis. The glitazones, troglitazone, rosiglitazone and pioglitazone inhibited proteoglycan biosynthesis by inhibiting glycosaminoglycan synthesis leading to shorter chains which showed reduced binding. There is little clinical evidence that sulfonylureas or biguanides reduce vascular disease whereas emerging evidence suggests that glitazones may have direct vascular actions to prevent lipid deposition and atherosclerosis. Our results indicate that the in vitro model of atherogenesis based of pharmacologically induced changes in PG biosynthesis may be useful for the evaluation of the vascular actions of new and emerging agents targeting vascular disease in diabetes. Our results indicate that the newest class of agents for the treatment of the high blood glucose of diabetes, the glitazones, also have the potential over and above the older drugs to have a more pronounced beneficial effect to prevent heart disease in people with diabetes. The project has been completed and submitted for publication.

Actions of calcium channel blockers on vascular proteoglycan synthesis: relationship to atherosclerosis

S Survase, M Ivey, J Nigro, N Osman and Peter J Little

Calcium channel blockers (CCBs) are a very widely used group of anti hypertensive agents. CCBs are efficacious in the reduction of blood pressure but the extent to which they manifest beneficial effects on cardiovascular disease is variable. Clinical studies indicate that pleiotropic actions make significant contributions to the efficacy of agents aimed at preventing atherosclerosis. The "response to retention" hypothesis implicates the binding and retention of lipoproteins by glycosaminoglycan chains on proteoglycans as an initiating step in atherogenesis. Atherogenic factors act as agonists and several classes of drugs including PPAR alpha and gamma ligands act as antagonists in this model. Initial data has demonstrated that high concentrations of CCBs inhibit proteoglycan synthesis. Newer preliminary data shows that the action is very modest at reasonable concentrations and appears to be independent of calcium channel blocking activity. We have reviewed the role of cardiovascular drugs acting on vascular smooth muscle proteoglycan synthesis and considered the potential action of CCBs in this model. We conclude that the inhibition of proteoglycan synthesis by CCBs does not play a role in the attenuation of atherosclerosis however, the anti-hypertensive efficacy and alternative pleiotropic actions provide support for the use of CCBs in the therapy of cardiovascular disease. The project has been completed and submitted for publication.

Grants and other Funding

- NHMRC Project Grant, Inhibition of c-Abl as a target for shortening glycosaminoglycan length on proteoglycans and preventing atherosclerosis. P Little, R Dilley.

- NHMRC Project Grant, Regulation of endothelin converting enzyme subcellular distribution and vascular endothelin production. I Smith, R Lew, W Thomas and P Little.

- Diabetes Australia Research Trust, Is an action of endothelin on vascular proteoglycans the link between endothelial dysfunction and accelerated macrovascular disease in diabetes? P Little.

- GlaxoSmithKline Australia Grant to support RMIT U Pharmaceutical Sciences Student, Mechanisms of atherosclerosis prevention with a focus on lower limb vascular disease. P Little.

Commercially Funded Research Activities

- Cryptome Pharmaceutical Ltd. P. J Little. Our laboratory supplied specialised cells (prepared in our laboratory) to the company for use in its drug development program.

Presentations

- Regulation of glycosaminoglycan synthesis by seven transmembrane receptor agonists. Baker Heart Research Institute, Research in progress Series Melbourne Australia. April 14th. Novel pathways regulating glycosaminoglycan elongation on vascular proteoglycans. Benaroya Research Institute, Hope Heart Program at Seattle Life Sciences and Swedish Hospital, Seattle WA USA. June 11th.

- Pathways to the prevention of atherosclerosis in diabetes: actions of PPAR alpha ligands on vascular proteoglycan biosynthesis and structure. Benaroya Research Institute, Hope Heart Program at Seattle Life Sciences and Swedish Hospital, Seattle WA USA. October 13th.

- American Diabetes Association, 64th Annual Scientific Meeting, Orlando, FL USA June 3-8. Little, P. J., M. Ivey and M. Hill. Endothelin 1 stimulates structural changes in vascular glycosaminoglycan chains which enhance LDL binding.

- P J Little. CVD in type 2 diabetes – combining evidence with science to reduce risk. Peter J. Little. Reducing Risk in T2D: A multi-dimensional approach to improve patient outcomes. A symposium on the occasion of the 40th Annual EASD Meeting Munich 2004. Chair: Professor Giancarlo Viberti.

- Mandy L. Ballinger 1,2, Julie Nigro 1,2, Thomas N. Wight 3 and Peter J. Little 1,2. Proteoglycans synthesized by Platelet Derived Growth Factor stimulated vascular smooth muscle cells show enhanced lipoprotein binding Gordon Research Conference – Proteoglycans July 11-16, 2004 Proctor Academy, Andover, New Hampshire, USA.

- Julie Nigro, Garry L. Jennings and Peter J. Little. AVBS Annual Scientific Meeting, Barossa Valley, SA, Australia. Saturday 11th September, 2004 Fenofibrate modifies human vascular smooth muscle proteoglycan synthesis and reduces lipoprotein binding

- Julie Nigro, Garry L. Jennings and Peter J. Little. ADS Annual Scientific Meeting, Sydney, Australia. Thursday 26th August, 2004 Fenofibrate modifies human vascular smooth muscle proteoglycan synthesis and structure and contributes to reduced lipoprotein binding.

- Ivey ME, Osman N and Little PJ. G protein coupled receptor agonists cause structural and synthetic changes in human vascular smooth muscle cells Australian Vascular Biology Society/Australian Atherosclerosis Society Joint meeting, Barossa Valley, South Australia, Australia. September 9 – September 12, 2004.

LIPOPROTEIN & ATHEROSCLEROSIS LABORATORY

Dmitri Sviridov

Head
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Research Projects

Hyperalphalipoproteinaemia and risk of atherosclerosis in heart transplant patients

D Sviridov, B Olchawa, A Hoang, J Chin-Dusting, P Nestel, A Dart

Heart transplantation has become a widely used procedure for the treatment of patients with end-stage heart failure. Heart transplant patients often have hyperalphalipoproteinaemia, which is not associated with decreased risk of CAD. Reverse cholesterol transport and endothelial function were compared in 25 transplant patients and 33 healthy controls. Transplant patients had significantly higher average HDL-C and apolipoprotein A-I levels, and both parameters were more widely spread than the control group. Transplant patients had higher levels of apoB and triglyceride, lower mass and activity of CETP respectively, lower mass of LCAT and higher activity of PLTP. The ability of plasma from patients to promote cholesterol efflux from cultured macrophages was lower compared to controls. When transplant patients were subdivided into two groups with high (HDL-C>1.4 mmol/L) and low (HDL-C<1.4 mmol/L) HDL, there was no difference in any parameter between the two groups. Forearm vascular responses to neither the endothelium dependent agonist acetylcholine nor the endothelium independent vasodilator sodium nitroprusside were influenced by HDL levels in the transplant patients. Lower LCAT and high PLTP are usually associated with low HDL-C while low CETP has an opposite effect. Together they may be responsible for greater variations in HDL levels in transplant patients. However, low LCAT and cholesterol efflux, high PLTP, triglyceride and apoB are all epidemiologically associated with increased risk of atherosclerosis.

Impact of HIV infection and treatment of AIDS patients with highly active retroviral therapy on reverse cholesterol transport

H Rose, J Hoy, A Dart, M Bukrinsky, D Sviridov

Both asymtomatic HIV and AIDS are consistently associated with higher risk of coronary artery disease (CAD). The major cause of CAD is atherosclerosis and a key element of atherosclerosis is the accumulation of cholesterol in the walls of arteries. This accumulation is caused by impaired metabolism of cholesterol. HIV patients have low levels of HDL and high level of triglyceride suggest that reverse cholesterol transport (RCT) pathway may be affected. Introduction of HAART resulted in a dramatic improvement in management of HIV, however the risk of CAD increased. Causes are not known. This year we contunued to study the effect of HIV and its treatment on intracellular cholesterol metabolism and reverse cholesterol transport. We found that a number of intracellular pathways are affected by HIV infection leading to accumulation of cholesterol in macrophages. We also found that reverse cholesterol transport is affected by HIV with the formation of dysfunctional HDL with impaired capacity to transfer cholesterol from cells to liver.

Effect of HIV infection on intracellular cholesterol metabolism

H Rose, M Bukrinsky, Dr. A Dart, D Sviridov

Both asymptomatic HIV-1 infection and AIDS are associated with increased risk of coronary artery disease (CAD). The major cause of CAD is atherosclerosis. A characteristic feature of atherosclerosis is accumulation of cholesterol-loaded macrophages ('foam cells') in the walls of arteries. Here we demonstrate that HIV-1 infection of macrophages leads to impairment of apolipoprotein A-I (apoA-I)-dependent cholesterol efflux, accumulation of cholesterol and formation of foam cells. This effect is mediated by the HIV-1 protein Nef. Indeed, Nef-deficient HIV-1 did not impair cholesterol efflux, while transfection of macrophages with the Nef-expressing construct resulted in reduction of efflux and cholesterol accumulation. One of the mechanisms responsible for this effect of Nef is reduction of expression and re-distribution of ATP-binding cassette transporter A1 (ABCA1), a main transporter of cholesterol to apoA-I. These results suggest a mechanism by which HIV-infected macrophages may initiate atherosclerotic plaque formation.

Effect of treatment with Crestor on parameters and functionality of reverse cholesterol transport

A Hoang, P Nestel, D Sviridov

A commercial project with Astra-Zeneka, the exact setup of the project is confidential.

Effect of transfection of macrophages with genes involved in cholesterol efflux and intracellular cholesterol metabolism

Y Fu, S Penfold, Z Krozowski, D Sviridov

In this project we propose to stimulate cholesterol efflux by activating or overexpresssing genes of the cholesterol efflux pathways and to measure cholesterol efflux and cholesterol accumulation in vitro and reverse

cholesterol transport and development of atherosclerosis in animal models of this disease. The long-term aim is to develop approaches to transfer these genes into human atherosclerotic plaque, preventing its progression and possibly stimulating its regression. Although overexpression of several individual elements of the cholesterol efflux pathway (e.g. ABCA1 or SR-B1) has been studied before, no attempts have been made to establish the most effective combination. It is becoming increasingly clear that cholesterol efflux pathways work in a tightly coordinated fashion supporting each other. Although eliminating rate-limiting step of one pathway would enhance cholesterol efflux it may lead to another step or a pathway becoming rate-limiting. We will attempt to use a combination of genes to eliminate as many rate-limiting steps as possible to develop the most universal approach.

ABCA1 expression in lymphocytes and muscle of athletes and patients with type 2 diabetes

C Treff, A Hoang, B Kingwell, D Sviridov

ABCA1-dependent pathway of cholesterol efflux consists of lipidation of apolipoprotein A-I (apoA-I) with cellular lipids and the formation of nascent HDL. In the absence of ABCA1 cells are unable to transfer lipids to lipid-free apoA-I with the formation of HDL. Mutations in ABCA1 cause Tangier's disease, a disorder characterized by the absence of HDL and non-existent reverse cholesterol transport. ABCA1 knockout mice show no plasma HDL, and accumulate cholesterol in macrophages. ABCA1 transgenic mice show increased levels of HDL, increased RCT, increased excretion of cholesterol and improved protection against atherosclerosis. It is therefore of paramount importance to be able to assess the levels of ABCA in humans. This project compared expression of ABCA1 in lymphocytes and muscle in individuals with very different levels of HDL. It will establish if there is a correlation between HDL-C levels and expression of ABCA1 in those tissues and if expression of ABCA1 in lymphocytes could be used as an indication of ABCA1 expression in other tissues.

Molecular mechanisms of involvement of Cholesteryl Ester Transfer Protein and Phospholipid Transfer Protein in lipid metabolism

U Tchoua, D Sviridov

A commercial project with Roche, the exact setup of the project is confidential.

Reverse cholesterol transport and type 2 diabetes

A Hoang, R O'Brien, D Sviridov

Diabetes is one of the most prevalent diseases in developed countries and the number of patients with diabetes is rapidly rising. Diabetes causes of a number of life-threatening complications and one of them is atherosclerosis. Accumulation of cholesterol in the vessel wall is a central event in the pathogenesis of atherosclerosis. One of ways to prevent development of atherosclerosis is to remove excessive cholesterol from the cells through reverse cholesterol transport pathway (RCT). The central element of this pathway is so-called high density lipoprotein (HDL). Patients with diabetes consistently have low levels of HDL, which may contribute to the development of atherosclerosis in diabetic patients. Objective of this study is to establish the relationship between diabetes and activity of RCT and HDL concentration. In this project we are probing plasma concentrations and activities of a number of lipoprotein sub-fractions, enzymes and transfer factors. Another focus of this project is the investigation of the effect of a number of factors found in the plasma of diabetic patients on intracellular cholesterol metabolism. Ultimately the aim is to identify new targets to increase HDL and decrease LDL-cholesterol thereby leading to new treatment strategies to prevent and retard cardiovascular complications in the high risk group of diabetic patients.

Grants and other Funding

- National Institutes of Health RO6, Impact of HIV infection and treatment of AIDS patients with protease inhibitors on reverse cholesterol transport. D. Sviridov (jointly with M Bukrinsky).

Commercially Funded Research Activities

- AstraZeneca Ltd, Defining the HDL-raising mechanism of rosuvastatin (Crestor) by quantitating the key steps of reverse cholesterol transport. P Nestel & D Sviridov.

- F Hoffmann-La Roche Ltd, Structure-function relationship of PLTP and CETP: Role in apoB secretion and cholesterol efflux. D Sviridov.

Presentations

- 5th Annual Conference on Arteriosclerosis, Thrombosis and Vascular Biology by AHA, 2004, San Francisco, CA, USA.

- Olchawa, B, Hoang, A, Schneider, L, Chin-Dusting, J, Kingwell, B, Nestel, P, Dart, A, Sviridov, D. Hyperalphalipoproteinaemia is Associated with Enhanced Reverse Cholesterol Transport in Athletes and Impaired Reverse Cholesterol Transport in Heart Transplant Patients.

- XV International Symposium on Drugs Affecting Lipid Metabolism, 2004, Venice, Italy.

- Escher, G., Krozowski, Z., El-Osta, A., Sviridov, D. Multiple gene overexpression leads to a dramatic increase of cholesterol efflux from macrophages.

ABMU & CENTRE FOR CLINICAL RESEARCH EXCELLENCE

Garry Jennings

COMMUNITY PROGRAMS

- The Alfred Baker Medical Unit (ABMU) is a collaborative research unit between the Baker and the Alfred that has been established for over 50 years. The unit provides a unique smooth interface between medical research and clinical research. It is a bridge between "bench top to bed side". This division conducts the preclinical and clinical trials of therapies developed in this and other Baker divisions, as well as those commissioned from outside. The Baker was the first Australian World Health Organisation Collaborating Centre for Research and Training in Cardiovascular Diseases.

- The Cardiovascular Disease Prevention Unit (CVDPU) is engaged in both domestic and international heart disease prevention projects. The CVDPU also coordinates, conducts and analyses major state wide, national and international clinical trials. A number of large international clinical trials were conducted at the Baker in 2004 including REACH and ON TARGET studies. Numerous smaller clinical trials were also carried out. The CVDPU was appointed by the Australian Society of Cardiothoracic Surgeons as a Data Management and Analysis centre for a project to identify key performance indicators for cardiac surgical outcomes. As a result of its demonstrated effectiveness in 2004, the program has expanded with other national centres joining the Baker in using these models to predict outcomes after cardiac surgery.

- The Risk Reduction Clinic is one way in which our expertise in reducing the risk of heart disease is made directly available to the community. The service is free of charge and is conducted by highly trained clinical nursing and technical staff. The staff at the Risk Reduction Clinic are involved in a broad range of research studies, including collecting samples for The Alfred & Baker Gene Bank, in addition to the critical role of recruiting subjects for ABMU studies. Recently, the Clinic has studied the genetic causes of hypertension and audited secondary prevention measures for heart attack and cardiac surgery patients. Research continues into better methods of defining risk in healthy subjects.

- The Baker is a World Health Organisation (WHO) Collaborating Centre for Research and Training in Cardiovascular Disease. The Appointment by the WHO to the Baker was the first of its kind in Australia. Currently, the Baker has two overseas projects with the WHO, one in Vietnam and one in Mongolia. The occurrence of heart disease in these and many Asian countries has escalated in the past few years mainly due to the erosion of traditional lifestyles with the increasing pervasion of Western influences. The joint WHO and Baker projects involve assessing the prevalence of heart disease in these countries and providing medical research training. This will enable the provision of better heart disease prevention, treatment and education in these regional countries.

- The Alfred and Baker Gene Bank is an important research initiative of the Baker Heart Research Institute. The aim of the Gene Bank is to collect samples of blood or tissue in order to study the genetic determinants of cardiovascular disorders. This research may lead to important new discoveries in drug treatment and prevention of heart attack and stroke. The Gene Bank relies on blood and tissue donations from healthy volunteer subjects in addition to people who have already had a heart attack, stroke or have high blood pressure, high cholesterol or other risk factors for cardiovascular disease, for example a family history of heart disease. Currently over 4000 volunteers have provided samples for the Gene Bank and it is well on the way to becoming an important resource for the discovery of new ways to treat and prevent heart disease.

If you would like further information on the ABMU, the Risk Reduction Clinic or the Gene Bank please call (03) 9276 2000.

CARDIOVASCULAR DISEASE PREVENTION UNIT

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Research Projects

REACH Study

C Reid, L Shiel, G Jennings

This study is an international prospective observational registry of subjects at increased risk of atherothrombotic events, coordinated in Australia by CVDPU together with the Department of Epidemiology at Monash University. The objectives of the study are to evaluate the long-term risk (yearly event rate) of atherothrombotic events globally, as well as in different population subgroups, compare outcomes within different subject profiles and define predictors of risk for subsequent events. In separate sub studies, data collected for the Australian cohort will provide information on the prevalence of peripheral artery disease (PAD) and provide estimates on the economic cost of clinical athero-thrombosis. The study will also enhance the quality of primary and secondary cardiovascular prevention among Australian general practitioners. The enrolment phase of the study was completed in July 2004 and recruited 2877 participants across Australia and over 50,000 patients worldwide, who will be followed-up for a period of 24 months.

Further updates from the 2nd Australian National Blood Pressure Study

C Reid, G Jennings

Following the publication of the main findings from ANBP2 in the New England Journal of Medicine in 2003, further work has focussed on a number of the sub-studies associated with the project. The diabetes outcome sub study showed that ACE inhibitor therapy was associated with a 34% reduction in new onset diabetes in comparison to diuretic therapy. This important finding may have a major influence for treatment choice for elderly patients with hypertension. Analyses from the Ambulatory blood pressure monitoring study have revealed that ambulatory blood pressure in a better predictor of cardiovascular events than clinica blood pressure in an elderly hypertensive population. In addition, it was found that night-time blood pressure was the most prominent predictor of cardiovascular events. This finding has major implications for the role of ambulatory blood pressure monitoring in the diagnosis and management of elderly hypertensive patients.

ONTARGET Study

G Jennings, C Reid, L Shiel, A Bruce, M Robotin

The Baker is the National Coordinating Centre or the ONTARGET a randomised multicentre international trial comparing the effectiveness of telmisartan (A-II antagonist), ramipril (ACE inhibitor) and their combination on outcomes on patients at high risk of cardiovascular disease. A parallel trial, TRANSCEND, is comparing the effects of telmisartan with placebo on outcomes of high-risk patients who are intolerant of ACE-inhibitors. Recruitment was completed in 2004, with over 30,000 patients recruited world-wide, who will be followed up for cardiovascular outcomes over a 5 year period. The BHRI is also involved in the Cardiac MRI and Ambulatory blood pressure sub-studies of ONTARGET, which enrolled 536 and 371 patients respectively.

ASPREE Study

C Reid, K. Murphy, L Shiel, M Nelson

The ASPREE (Aspirin in reducing events in the elderly) study is a placebo-controlled trial of low-dose aspirin for the primary prevention of major cardiovascular events and vascular dementia in participants over 70 years of age. As gastrointestinal bleeding and intracranial haemorrhage are known side effects of aspirin treatment, the study will also ascertain the balance of risks versus benefits of aspirin treatment in this patient group. The pilot phase of the study is nearing completion and based upon its success, the study has attracted NHMRC support to enrol 15,000 subjects, to be followed up for an average period of 5 years.

UNIVERSE Study

L Shiel, M Robotin, C Reid

UNIVERSE (RosUvastatiN Impact on Ventricular Remodelling lipidS and cytokinEs) is a double-blind, randomised, placebo controlled, multicenter, phase III study aiming to assess the impact of rosuvastatin treatment on left ventricular function, cytokines and lipid parameters in patients with established systolic chronic heart failure. CVDPU performs the Data Management role for the study, which enrolled 131 patients in

Australia, in 19 study centres. Patient follow up will be completed in 2005.

HYVET study

Colin Johnston, Chris Reid, Ann Bruce

The Hypertension in the Very Elderly Trial (HYVET) is a randomised double-blind trial designed to detect a difference in stroke events between placebo and active treatment (diuretic and ACE-inhibitor) in patients aged 80 years and over. Recruitment for the trial commenced in late 2004 and is expected to be complete by mid-2005.

Ambulatory Blood Pressure Monitoring Study

C Reid,G Head, L Shiel

Using a prospective clinical trial design, this project aims to identify predictors of early morning surge in BP in hypertensive versus normotensive groups. Volunteer subjects presenting for clinical diagnosis of hypertension and those attending a community based risk factor screening clinic will undergo 24 hour ambulatory blood pressure monitoring (ABPM) and provide additional information regarding their lifestyle and medical history. Subjects will be monitored annually over a three-year period at which all baseline measurements will be re-assessed. The predictors of the rate of rise and fall of BP and HR in normotensive and hypertensive patients will be determined using multivariate analysis. On the basis of the success of its pilot phase, the project has attracted NHMRC endorsement and will be scaled up in 2005.

Primary prevention of CVD in general practice (cvTRACplus / Heart Care)

C Reid, C Retegan

CVDPU has developed computer based data collection and reporting programs to assist GPs identify and manage risk factors for cardiovascular disease in general practice. The program has been successfully running for over 6 years, with over 25,000 patients having had over 60,000 study visits during this time period. Data collection will be completed in 2005 and it is expected that the data analysis will provide new insights into the prevalence and distribution of cardiovascular risk factors in the general population, as well as on the proportion of patients receiving medical treatment for dyslipidemia, hypertension and diabetes.

Secondary prevention of CVD (GMHBA)

S Birrell, P. Davern, C Reid

The project aims to improve the secondary prevention of cardiovascular disease in patients following a diagnosed cardiovascular event (myocardial ischaemia or stroke). Patients were invited to enrol by their health insurance provider, a not-for-profit organisation based in Geelong, GMHBA. Patients wishing to enrol and their general practitioners provided relevant information for the determination of a risk profile. Based upon this information, a computer assisted decision support system developed at the Baker Institute generated individualized evidence-based recommendations to patients and their doctors. The study uses methodology validated by the SENTINEL program, which successfully demonstrated that computer-generated, individualized patient recommendations increase the likelihood of patients achieving target lipid levels and increased patient compliance with medications.

FIST study

J Chellapah, A Tonkin, C Reid

This study involved the participation of both parents and schools in an intervention providing healthy eating information and serves of fruit daily in the classroom, coupled with an assessment of children's dietary choices using 3-day weighted food diaries, food frequency and lifestyle questionnaires. Body mass index, lipid and glucose levels measurements and an assessment of students' fitness levels were measured at enrolment and upon completion (8 weeks later), to evaluate changes in adiposity and cardiovascular risk factors attributable to the intervention. The pilot phase of the study has been successfully completed and it is envisaged that the study will be rolled out in several schools in the Melbourne area during 2005.

ASCTS Database Project

S Birrell, P Davern, A Nadonza, C Reid

The Victorian Division of the Australian Society of Cardio-thoracic Surgeons appointed the Baker Institute to act as the Data Management and Analysis centre for a project aiming to identify key performance indicators for cardiac surgical outcomes. A standard database was developed for the project, and adopted by all public hospitals performing cardiac surgery in Victoria. 2004 saw the development of risk-adjusted outcome models measuring surgical performance, which will make possible the benchmarking of surgical performance nationally and well as internationally. As a result of its demonstrated effectiveness, there is a high level of interest in the project from other centres and it is being envisaged that the program will be expanded nationally over the next few years. During 2004, one surgical group in Queensland joined the program and the NSW public and private hospital groups are expected to join the group in 2005.

MVSA project

C Retegan, C Reid, M Robotin

In 2004, the Melbourne Vascular Surgeons' group (MVSA) appointed the CVDPU as its data management centre and for conducting clinical audit activities for all 13 surgical units based in Victorian public hospitals. A series of 5 key performance indicators were used to benchmark the performance of surgical units and formed the basis of a

comprehensive data analysis and reporting system. The CVDPU brief includes the performance of clinical audits, data analysis and the production of individual unit and surgeon reports. Pending the successful completion of this pilot phase, it is being envisaged to expand the program to all participating hospitals in the following year.

Grants and other Funding

- National Heart Foundation.
A randomised controlled trial on aspirin in the primary prevention of CVD events in the elderly ASPREE. C Reid, M Nelson, H Krum, C Johnston

Commercially Funded Research Activities

- Sanofi-Synthelabo, REACH Registry. C Reid, L Shiel, M Robotin.

- MSD Australia, cvTRAC/ Heartcare. C Reid, C Retegan
- BMS Australia, Sentinel. C Reid, S Birrell.

- GMHBA , secondary CV prevention. C Reid, S Birrell, P Davern, A Nandonza.

-nDept Health Victoria, ASCTS Database Project. C Reid, S Birrell, P Davern.

- Melbourne Vascular Surgeons' Association, MVSA database project. C Retegan, C Reid, M Robotin.

Invited Presentations

- Colorectal Cancer National Database Workshop. Data Collection and management - Cardiac Surgery, Melbourne, May, 2004 (C. Reid).

- Questionnaire and clinical record form design. Data collection and analysis for small clinical studies workshop. Melbourne, May 2004. (L Shiel).

- Clinical Registries Workshop. Governance Issues in the establishment of Clinical Registries, Melbourne, November 26th 2004 (C. Reid).

- Cases, Controversies and the Cutting Edge in Cardiology II, Epidemiology and Clinical Trials, Melbourne, November 19-21st , 2004 (C. Reid).

Conference Presentations

- Kingwell B, Dart AM, Cameron JD, Liang YL, Berry K, Gatzka C, Reid CM, Jennings GL. Cholesterol and large artery stiffness in a hypertensive population. J Hypertension 2004, 22 (S1); S168.

- Wing LMH, Reid CM, Ryan P, Beilin L, Brown M. Outcome in relation to clinic and ambulatory blood pressure in the 2nd Australian National Blood Pressure Study. J Hypertension 2004, 22 (S1); S58.

- Wing LMH, Reid CM, Ryan P, Beilin L, Brown M. Outcome in relation to clinic and ambulatory blood pressure in the 2nd Australian National Blood Pressure Study. AHMRC Conference Proceedings, 2004: 136. ISSN:1447-60140.
- Chellappah, JM, Reid CM, Tonkin A. A clustered randomised controlled school community intervention to improve dietary habits and cardiovascular risk factors of children and young adults – pilot study. AHMRC Conference Proceedings, 2004: 245. ISSN:1447-60140.

- Burke K, Dart AM, Reid CM, Jennings GL, Williams C. The Alfred Baker Gene Bank- facilitating diverse genetic approaches for the fight against cardiovascular disease. AHMRC Conference Proceedings, 2004: 329. ISSN:1447-60140.

- Reid CM, Skillington P, Shardey G, Birrell S, Smith J, Yii M, Seevanayagam S, Mohajeri M, Pick A. Monitoring Unit and Individual Surgeon Performance with the ASCTS Database. ASCTS ASM Handbook 2004; 124.

- Reid CM and Thrift AG. Hypertension 2020 – confronting tomorrow's problem today. Proceedings of the Long term control of blood pressure conference 2004.