Cardiovascular Research at the Baker Heart Research Institute – Part 1

This year marks the 80th anniversary of the founding of the Baker Heart Research Institute at the Alfred Hospital campus in Prahran, Melbourne. The Baker Institute, named after its benefactors Thomas and Alice Baker, originally focused on providing medical pathology services to the Alfred Hospital and in 1938 commenced its focus on physiology and experimental medicine. In 1975, with the appointment of Professor Paul Korner as Director, the Baker’s focus became solely cardiovascular, and it received national and international recognition for its leadership on research into hypertension and heart disease. In 2001, the Baker moved to a new 8 level premises, striking for its Piet Mondrian-inspired exterior colour scheme and four-level atrium. Built as part of the Alfred Medical Research and Education Precinct opposite Fawkner park, this new site provides one the finest research facilities of its kind in Australia with six specialised laboratory floors, a state-of-the-art DNA sequencing service, and a “Ramaciotti Foundation” funded Proteomics facility. There are also two floors for biotechnology companies and a close connection to the Alfred Baker Medical Unit and Heart Centre, which are located within the Alfred Hospital. In 2001, Professor Garry Jennings became Director of the Institute after being Deputy Director and head of the Alfred’s Heart Centre.

The Institute now consists of over 250 staff and students organised into four major divisions which are; the Experimental Cardiology and Heart Failure Division headed by David Kaye, the Atherothrombosis and Vascular Division headed by newly arrived from Germany, Dr. Karheinz Peter, the JDRF Diabetes & Metabolism Division headed by Professor Mark Cooper and the Cardiovascular Neurosciences Division headed by Professor Murray Esler.

This newsletter is Part 1 from the Baker Institute and comprises some of the research areas related to hypertension and cardiovascular disease.

Wynn Department of Metabolic Cardiology: Professor David Kaye

In 2003, David Kaye was appointed as Head of the Wynn Department of Metabolic Cardiology, which was established through a generous grant from the Atherosclerosis Research Trust of the United Kingdom. Some of Prof. Kaye’s recent research work has been directed at exploring the role of impaired L-arginine transport in the pathogenesis of endothelial dysfunction in disease states including atherosclerosis, heart failure, diabetes and hypertension.
**Arginine studies**  
Samara Finch, David Kaye and Zhiyong Yang have been examining the molecular basis for abnormal arginine transport in essential hypertension. Previously, we had reported the identification of 13 single nucleotide polymorphisms (SNPs) around the CAT1 gene. Among them, one SNP at 3’ untranslated region (UTR) was of great interest in terms of its potential role in gene regulation in transcriptional level, we therefore surveyed the allele frequency of this SNP with DNA samples deposited in Baker-Alfred’s Gene Bank Collection from people with or without hypertension. We found that 3’ UTR polymorphism may play a role in regulating CAT-1 gene expression, thus regulating arginine transport. Zhiyong Yang is now developing a CAT1 transgenic mouse.

Wei-Zheng Zhang has found that a cigarette smoke extract is able to chemically modify active molecules to affect endothelial arginine uptake and arginine metabolism resulting in decreasing intracellular arginine bio-viability, reducing nitric oxide production and increasing asymmetric dimethylarginine which is a new risk factor for cardiovascular diseases.

David Kaye and his team are identifying novel modulators of arginine transport and have screened a library of 300 novel compounds for their ability to increase intracellular arginine transport. In recent studies they found that some of the most promising compounds lowered blood pressure. Other projects include the role of oxidative stress, angiotensin peptides and insulin in regulating arginine transport. A major undertaking has been determining the relevance of arginine metabolism to patients with Coronary Artery Disease by assaying samples from over 200 subjects.

**Chronic Heart Failure (CHF)**
The other major focus of the laboratory is to understand the neurobiological and metabolic mechanisms in heart failure. A characteristic of heart failure is increased activity of the sympathetic nervous system (SNS), presumably to compensate for a reduction in cardiac output and to maintain tissue perfusion. Increased SNS activity commonly results in excess norepinephrine remaining within the myocardium. Tanneale Marshall and David Kaye are generating a recombinant adenovirus containing the norepinephrine transporter (NET) gene. The aim of this study is to determine whether expression of NET in cardiomyocytes can reduce the cardiotoxic affects of excess NE associated with heart failure. Little is known or documented about the mechanisms controlling NETs trafficking to the cell membrane or the biochemical pathways it signals through. We have been able to demonstrate that mutations within the Grb2 consensus sequence do not affect RNA synthesis (conventional RT-PCR) or protein synthesis (western blot analysis). Through the application of fluorescent confocal microscopy and western blot analysis of isolated cell membranes, we have shown that these mutations do not alter localisation of NET to the membrane.

Samara Finch and David Kaye are currently examining the role of atrial fibrillation in heart failure. Previous research has shown that baroreceptors are blunted in CHF and that this is associated with prognosis. Previously, we have shown that sympathetic activity is not further elevated with atrial fibrillation in CHF at rest in a well treated group of patients, but the affect atrial fibrillation has on baroreceptor response in CHF and on sympathetic response to exercise is unknown. Our studies have shown that AF further impairs sympathetic response to baroreceptor unloading in CHF and postulated that the mechanism is secondary to atrial fibrosis. Subsequent to this, we collected right atrial appendages from cardiac surgical patients (11 in atrial fibrillation and 11 in sinus rhythm) and have shown significant increases in collagen content in atrial fibrillation patients by Sirius red staining. To further extend the research into the haemodynamic impairment and outcome of heart failure patients with atrial fibrillation a Real Time RT-PCR method was developed to examine the mRNA expression profile of Nerve Growth Factor (NGF) in atrial fibrillation and SR. NGF is essential for the development and maintenance of sympathetic neurons.

**Recent Publications**
Coronary heart disease and heart failure remain the major cause of mortality in Australia, much of which is a consequence of either cardiac hypertrophy, reperfusion injury, and/or cardiovascular complications of diabetes. Studies in Dr Rebecca Ritchie’s Molecular Pharmacology laboratory at the Baker Heart Research Institute in Melbourne continues to focus on identifying novel therapeutic strategies to minimise structural and functional damage to the heart inflicted by these pathological disorders. Targeting myocardial oxidative stress, from the cellular/molecular level to the intact heart, is the underlying theme of this research. We have identified a number of endogenous (intra)cellular antihypertrophic mediators, including bradykinin, nitric oxide, cyclic GMP, and natriuretic peptides. In particular we recently identified that atrial natriuretic peptide (ANP) suppresses cardiomyocyte reactive oxygen species generation as part of its antihypertrophic actions (see Figure). We have also recently demonstrated that the endogenous anti-inflammatory protein annexin-1 and the red wine constituent resveratrol represent novel cardioprotective mechanisms: both agents protect the heart under conditions of ischaemia-reperfusion injury in vitro; post-ischemic contractile dysfunction and cardiac myocyte injury are significantly reduced, as is formation of reactive oxygen species.

Abnormalities in myocardial function, particularly diastolic dysfunction, and the cardiac hypertrophy and fibrosis (see Figure) that can underlie these functional problems, are characteristic of the diabetic heart, and likely contribute to the high incidence of cardiovascular mortality in diabetic Australians.

We have evidence that increased steady-state levels of reactive oxygen species, from NADPH oxidase and other sources (mitochondria, uncoupled nitric oxide synthase, NOS), is a contributing mechanism to these diabetic cardiac complications in vivo. Thus, our ongoing studies across cardiomyocyte hypertrophy, ischaemia-reperfusion, and the diabetic heart suggest that administration of effective reactive oxygen species-limiting therapies, alone or on top of current therapy, will likely have incremental cardiac benefit in affected patients, limiting progression to heart failure and mortality.

Recent Publications
Anti-platelet therapy and anticoagulation are the cornerstones of the acute treatment as well as the prevention of myocardial infarction. However, limited efficiency and adverse effects, in particular bleeding complications, have prompted intensive search for better therapeutic agents. Professor Karlheinz Peter heads a newly created Centre for Thrombosis & Myocardial Infarction at the Baker Heart Research Institute in Melbourne.

The Centre spans from basic research in molecular biology to clinical trials in cardiology. This includes studies on the cellular mechanisms of coronary artery disease, encompassing the role of platelets, coagulation and inflammation in atherosclerosis, as well as the mechanisms leading to the rupture of atherosclerotic plaques. One of Karlheinz’s major goals is the development of so-called “intelligent” drugs, tailored to specifically block only activated platelets in the circulating blood, or to provide anticoagulation only at the clot, the site where it is needed most. This approach is based on single-chain antibodies that were generated from human single-chain antibody phage libraries. These recombinant antibody fragments are directed against epitopes, which are only present on the activated GPIIb/IIIa receptor and are thus specific for activated platelets. Thereby, the treatment and/or prevention of myocardial infarction and stroke should be more efficient and the risk of bleeding complications should be reduced markedly. The first promising agents have already been developed within the Centre.

Karlheinz obtained his MD and PhD at the University of Freiburg, Germany, where he also spent several years in clinical practice as a cardiologist, most recently as the head of the cardiac catheter laboratory. He worked as post doctoral research officer at the Johns Hopkins University, Baltimore, USA and at the Scripps Research Foundation, San Diego, USA. Most of his clinical training was done at the University of Heidelberg, Germany. Karlheinz was attracted to the Baker Institute by its unique combination of clinical and basic research, made possible by the partnership with The Alfred Hospital, Monash University, and the Australian Centre for Blood Diseases.

Recent Publications
3. Kanse SM, Matz-Westphal R, Preissner KT, Peter K. Vitronecin is a ligand for the b2 integrin Mac-1 (αMβ2, CD11b/CD18) and this interaction promotes leukocyte adhesion. Arteriosclerosis, Thrombosis and Vascular Biology 2004;24:2251-6
**Molecular Endocrinology Laboratory: Dr Walter Thomas**

Our aim is to understand the processes that allow membrane receptors to transmit signals and affect cell function, including the mechanisms used by cells to limit signalling and permit responses of appropriate strength and duration. Specifically, our research focuses on seven transmembrane spanning, G protein-coupled receptors (GPCRs) that represent the largest receptor superfamily in our genome. Mutations and modifications of GPCRs and their signalling and regulatory partners are linked to dysfunction and disease and drugs that target these receptors are prime candidates for therapy. We study the activation and deactivation of two main, clinically relevant GPCRs – the angiotensin type 1 receptor and urotensin II receptor. Perturbations in these systems are linked to hypertension and cardiovascular disease and significant insights into the biology of receptor systems have already impacted clinical practice (eg, the use of non-peptide AT1 receptor antagonists to alleviate high blood pressure and reduce mortality).

Our research group consists of post-doctoral fellows, research and technical assistants, and PhD and honours students (see photo) and funded collaborations with Ross Hannan, Mibel Aguilar, Andrew Allen, and Stephen Harrap (et al.).

Our research efforts are aligned to the four NHMRC project grants currently held, particularly my two CIA NHMRC projects. One is a 5-year project grant based on our discovery that angiotensin receptor activation induces cardiac cell growth and signalling via trans-activation of the epidermal growth factor receptor. Our research dissects and understands EGFR trans-activation from a molecular, cellular and in vivo perspective. Outcomes will provide important information on, and potential targets for, left ventricular hypertrophy – a major independent risk factor for cardiac failure and death. The other 3-year project grant focuses on the molecular and cellular processes that dictate activation and trafficking of regulatory and scaffolding proteins termed arrestins, as well as the contribution of arrestins to the complexity of GPCR function. Outcomes from these studies will offer new possibilities for blocking/augmenting GPCR actions – particularly, angiotensin and urotensin receptors that contribute significantly to human health and disease.

**Neuropharmacology Laboratory: Associate Professor Geoffrey Head**

Increased activity of the sympathetic nervous system (SNS) occurs in both hypertension and congestive heart failure. The focus of the Neuropharmacology Laboratory, led by Geoff Head, (NHMRC Principal Research Fellow), is to understand how brain pathways and neurotransmitters regulate the cardiovascular system and to discover the mechanisms responsible for the overactivity of the SNS in these diseases. An understanding of this process may reveal how the SNS may contribute to the long term regulation of blood pressure.

**Role of the SNS in Angiotensin Dependent Hypertension**

We have found that centrally acting antihypertensive drugs that inhibit the SNS are much more effective in normalising blood pressure in this form of hypertension, suggesting an overactive SNS. This is supported by our recent evidence that ganglionic blockade produces a much greater fall in blood pressure in hypertensive animals. However, directly recorded renal sympathetic nerve activity is not elevated when the hypertension is established. This may be explained by a greater neuroeffector response, an altered pattern of sympathetic activity or possibly a contribution from vascular remodeling. Sandra Burke and MSc student John-Luis Moretti are determining the importance of the renal nerves and the role of vascular hypertrophy, in the development of angiotensin dependent hypertension. Geoff and Sandra have been collaborating with Roger Evans from Monash University and Gavin Lambert, to investigate whether this paradox may be explained by an increased neuroeffector mechanism in the kidney. Pamela Davern, recently arrived from the Howard Florey Institute and currently completing her PhD, has used immunohistochemical methods with Fos related antigen (FRA), to investigate brain regions responding to long term activation by angiotensin and to identify nuclei that may be relevant to sympathetic nerve activation.
Central Cardiovascular Pathways involved in stress and L-NAME induced hypertension

Having established a role for central angiotensin and its subsequent activation of hypothalamic and brainstem superoxide cell signaling during stress, Dmitry Mayorov, HBPRCA Fellow, Honours student Daian Chen, and Geoff Head are now working to determine the sources of superoxide production, the downstream products and the signaling pathways. They also plan to explore how these systems contribute to the long term adaptive changes that occur with chronic emotional stress using AT1 receptor knockout mice. There is evidence that increased activity of NADPH oxidase at the level of pre-sympathetic nuclei of the hypothalamus and brainstem contributes to the maintenance of L-NAME-induced hypertension. James Armitage, recently arrived after 3 years in London as a Postdoctoral Research Fellow, Maternal and Fetal Research Unit at King’s College, is examining the effect of chronic treatment with L-NAME on BP in order to assess the contribution of the SNS to the hypertension. They will now determine the expression of gp91phox and p47phox subunits of NADPH oxidase in the hypothalamus and rostroventrolateral medulla.

Cardiovascular effects of Taipan natriuretic peptides

Natriuretic peptides which act in the body to oppose the activity of the renin-angiotensin system and enhance excretion of sodium and water, are found in many species. In collaboration with Professor Alewood at the Institute for Molecular Bioscience University of Queensland we have examined a series of peptides isolated from the venom of the Taipan which have activity similar to the human natriuretic peptides. Geoff Head and Lihong Kong, who recently completed her PhD at University of NSW, have been characterizing the cardiovascular actions of these peptides and a number of newly synthetized analogues in conscious rabbits and in heart failure rats. This project has become a commercial development with the formation of a new spin-off company Elacor.

Cardiovascular Profile Of Aromatase (Estrogen Deficient) Mice

There has been much interest in the cardiovascular role of estrogens with the consistent findings that men and postmenopausal women are at higher risk of developing cardiovascular disease than premenopausal women. We now have access to mice lacking estrogen because the enzyme aromatase has been knocked out and have shown they have lower blood pressure and baroreflex sensitivity. In a collaboration with Margaret Jones at Monash University, Honours student Thu-Phuc Nguyen-Huu will examine the effect of a high salt diet and estrogen replacement on these mice using radiotelemetry to measure blood pressure, as estrogen may be protective against the adverse effects of a high salt diet.

Morning Surge In Blood Pressure as a Risk Factor In Cardiovascular Disease

It is well recognised that the occurrence of strokes and cardiac infarcts in humans is highest in the morning when blood pressure is rising from low overnight values. Geoff and Elena Lukoshkova, a scientist from Moscow, have developed a logistic curve fitting procedure which measures the diurnal rates of transition in cardiovascular variables and from the active to the asleep periods separately. As part of a larger collaborative effort with Barry McGrath (Dandenong Hospital) and Chris Reid, Melinda Carrington, (PhD pending), and Honours Student Nicola Fotheringham are characterising predictors of the rate of morning rise in blood pressure and heart rate in a large patient group to determine whether subjects with an excessive morning surge have altered functioning of the SNS and whether there is a relationship with vascular compliance.

Aetiology of Obesity Related Hypertension

Up to 70% of newly diagnosed hypertension in the Framingham study is attributed to obesity, and study of obesity and its related cardiovascular risk is an emerging focus. Geoff and Nina Eikelis have shown that obese rabbits are resistant to the central effects of leptin in relation to satiety but not with regard to SNS activation. This suggests that leptin resistance at the level of the hypothalamus is selective to appetite regulation and that continued stimulation of SNS vasomotor tone may drive the hypertension observed with obesity. James, Geoff, Luisa LaGreca and Genevieve Martin (RMIT Pharmaceutical Science student) are examining the neurogenic mechanisms underpinning the development of obesity related hypertension in rabbits by characterising changes in CNS pathways that modulate SNS activation (by FRA immunohistochemistry and targeted blockade of hypothalamic nuclei) early during
the consumption of a high fat diet. We believe that changes in sympathetic vasomotor tone may occur very early during the development of obesity and that the selective CNS changes that result in this neurogenic form of hypertension may be pivotal in the development of obesity related cardiovascular disease.

**Recent Publications**