

MONASH UNIVERSITY
DEPARTMENT OF PHARMACOLOGY
HONOURS PROJECTS 2010 (Oct 15, 2009)

Labs/ Supervisor(s)	Project Title
Drug Discovery Biology Labs: (Parkville) Christopoulos & Leach Christopoulos & Valant	<ul style="list-style-type: none"> • Allosteric modulation of the extracellular calcium sensing receptor • Engendering biased signalling using bitopic orthosteric/allosteric ligands of GPCRs
Venoms & Toxins Labs: Hodgson et al	<ul style="list-style-type: none"> • Solving the 'Brown snake paradox' • New toxin classes from the venoms of Anguimorpha lizards
Vascular Pharmacology & Immunobiology Labs: Miller/Kemp-Harper Broughton/Sobey Miller/Sobey	<ul style="list-style-type: none"> • Exploring the vasoprotective actions of nitroxyl (HNO) • Role of G-protein oestrogen receptor in the brain following stroke • Investigating the role of NADPH oxidase in cerebral artery dysfunction following stroke
Integrative Cardiovascular Pharmacology Labs Gaspari/Widdop Jones/Widdop	<ul style="list-style-type: none"> • AT₄ receptor/IRAP as a target for protection in cardiovascular disease? • Cardiovascular effects of novel AT₂ receptor ligands
Baker IDI (Pahran) Andrews/Kemp-Harper/Chin-Dusting Ritchi/Irvine/Kemp-Harper Ritchie/Bowden/Kemp-Harper	<ul style="list-style-type: none"> • A role for nitroxyl (HNO) in the treatment of nitrate tolerance in human blood vessels? • Novel activators of soluble guanylate cyclase as new treatments for cardiac hypertrophy and fibrosis • Reactive oxygen species (ROS) induced damage in the diabetic heart: mechanisms, consequences and prevention

For more details, contact Hons Convenor: robert.widdop@med.monash.edu.au

Allosteric modulation of the extracellular calcium sensing receptor.

Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences

Supervisors: Prof. Arthur Christopoulos (arthur.christopoulos@med.monash.edu.au) and Dr. Katie Leach (katie.leach@med.monash.edu.au)

G protein-coupled receptors (GPCRs) represent the major single class of all drug targets. Traditional approaches to GPCR-based drug discovery have focused on optimizing the interaction of potential drug candidates with the receptor's *orthosteric* site, i.e., the binding site utilized by the endogenous agonist; nearly all synthetic agonists and competitive antagonists currently on the market fall under this category. However, there still remains a significant and unmet need for better selectivity and a lower side-effect profile for the treatment of many disorders associated with GPCR dysfunction.

One approach to addressing the drug selectivity issue is to target novel ligands to *allosteric* binding sites on GPCRs, which are topographically distinct from the more conserved orthosteric site. The extracellular calcium sensing receptor (CaSR) is the first GPCR for which an allosteric drug has made it to the market (2004; for the treatment of hyperparathyroidism). Cincalacet (Sensipar) is a positive allosteric modulator, which potentiates the actions of circulating extracellular calcium to normalize parathyroid hormone levels. However, the CaSR is also known to be subject to nearly 200 different naturally occurring mutations and polymorphisms, many of which are implicated in disease. The impact of these mutations on allosteric drug therapy at this receptor remain largely unknown.

The current project will thus investigate the effects of a variety of positive and negative allosteric modulators on the signaling of the wild type and selected, clinically relevant, mutations of the CaSR. This knowledge will eventually allow for a more personalized approach to treating disease associated with the CaSR.

Techniques that will be used in this project include:

Structural: Site-directed mutagenesis and ligand-directed molecular modelling, to develop a better picture of the CaSR structure and to identify any potential "druggable" pockets.

Pharmacological: Receptor-G protein interaction will be quantified by downstream signalling using state-of-the-art, high throughput assays of extracellular signal regulated kinases (ERK1/2), and intracellular calcium mobilization.

Imaging: If time permits, you will also monitor the trafficking and distribution of the receptors by either confocal microscopy or state-of-the-art high content imaging.

Molecular biological: As part of the project you will also learn basic molecular biological procedures including isolation and preparation of DNA, cell culture and cell transfection.

Engendering biased signalling using bitopic orthosteric/allosteric ligands of GPCRs.

Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences

Supervisors: Prof. Arthur Christopoulos (arthur.christopoulos@med.monash.edu.au) and Dr. Celine Valant (celine.valant@med.monash.edu.au)

Allosteric modulation of G protein-coupled receptors (GPCRs) represents an exciting approach to drug discovery by targeting sites that do not display the same degree of high conservation across receptor subtypes as does the orthosteric site (the site utilized by the receptor's endogenous agonist). Allosteric modulators are ligands that bind to allosteric sites and modulate the activity of an orthosteric agonist, either in a positive or a negative direction. Based on our most recent studies of the muscarinic acetylcholine receptor (mAChR) family of GPCRs, we have identified a new mode of GPCR drug action, which we have termed "bitopic", i.e., the *same* ligand possesses *both* an allosteric *and* an orthosteric moiety that simultaneously engage both sites on the receptor. Furthermore, the allosteric part of the molecule changes the signalling efficacy of the orthosteric part of the molecule. We hypothesize that this mechanism of action can explain differences in the pharmacological profile of certain agonists that display functional selectivity ("biased signaling") for a given receptor subtype of others of the same family.

To test this hypothesis, the current project will investigate the pharmacology of novel bitopic ligands of the muscarinic and adenosine family of receptors, and compare this to the actions of the individual orthosteric and allosteric fragments that comprise the bitopic molecules.

The outcome of these studies will determine whether our proposed mechanism(s) of allosteric modulator-engendered signaling bias is a general phenomenon applicable to multiple GPCRs.

Techniques that will be used in this project include:

Pharmacological: Receptor-G protein interaction will be quantified by measuring [³⁵S]GTP γ S turnover and, downstream signalling will be monitored using state-of-the-art, high throughput assays of extracellular signal regulated kinases (ERK1/2) and intracellular calcium mobilization.

Molecular biological: As part of the project you will also learn basic molecular biological procedures including isolation and preparation of DNA, cell culture and cell transfection.

References:

1. Valant, C, Sexton, P.M. and A. Christopoulos (2009) Orthosteric/Allosteric Bitopic Ligands: Going hybrid at GPCRs. *Molec. Interventions*, **9**: 125-135. *Cover article*.
2. Valant, C., Gregory, K.J., Hall, N.E., Scammells, P.J., Lew, M.J., Sexton, P.M. and A. Christopoulos (2008) A novel mechanism of G protein-coupled receptor functional selectivity: muscarinic partial agonist McN-A-343 as a bitopic orthosteric/allosteric ligand, *J. Biol. Chem*, **283**: 29312-29321. (See also "Research Highlights", *Nature Rev. Drug Discover.* (2008) **7**: 976).

HONOURS PROJECT 2009

Solving the “Brown snake paradox”

Supervisor: A/Professor Wayne Hodgson

Co-supervisor: A/Professor Geoff Isbister (Menziess School of Health Research, Darwin)

The Eastern Brown Snake (*Pseudonaja textilis*) is one of the world’s most venomous snakes with a murine LD₅₀ value of 0.04 mg/kg (s.c.) (Broad et al., 1979) ranking it #2 behind the Inland taipan (*Oxyuranus microlepidotus*) and just in front of #3 the Coastal taipan (*O. scutellatus*). All three venoms contain highly potent presynaptic (β)-neurotoxins i.e. textilotoxin, paradoxin and taipoxin, respectively (Su et al., 1983; Hodgson et al., 2007; Fohlman et al., 1976). However, while the taipans cause marked neurotoxicity in envenomed humans, bites by brown snakes are notable for the absence of neurotoxicity. This is often referred to as the ‘brown snake paradox’. We hypothesize that the absence of neurotoxicity in brown snake bites is due to only small amounts of the potent textilotoxin being present in the venom.

This study will compare the *in vitro* neurotoxicity of venom from brown snakes (*P. textilis*, *P. nuchalis*, *P. affinis*) with that of the taipans (*O. scutellatus*, *O. microlepidotus*, *O. s. canni*). The pre-synaptic neurotoxins will be isolated from the venoms using size-exclusion HPLC, and the quantity and neurotoxicity of each β-neurotoxin compared to estimate the potential potency of the venom versus the potency of the toxins.

Table 1 Some representative presynaptic (β)-neurotoxins isolated from snake venoms

Toxin	Subunit composition	Common name	Snake		References
			Scientific name		
Notexin	Single chain	Australian tiger snake	<i>Notechis scutatus</i>		73
Taipoxin	Three subunits	Australian coastal taipan	<i>Oxyuranus scutellatus</i>		24, 26, 72
Paradoxin	Three subunits	Australian inland taipan	<i>Oxyuranus microlepidotus</i>		27, 71
Crotoxin	Two subunits	South American rattlesnake	<i>Crotalus durissus terrificus</i>		24, 26, 70, 74
Textilotoxin	Four subunits	Australian common brown snake	<i>Pseudonaja textilis</i>		14, 28
β-Bungarotoxin	Two subunits*	Asian krait	<i>Bungarus multicinctus</i>		24, 26, 74

*Covalently linked.

Table from

Hodgson & Wickramaratna (2002)

The aim is to determine if the potency of brown snake venoms is similar or less than taipan venoms in a neurotoxicity assay to attempt to explain the lack of neurotoxicity in envenomed patients.

References

- Broad, A.J., Sutherland, S.K. & Coulter, A.R. *Toxicon* 17, 661–664, 1979.
 Fohlman, J., Eaker, D., Karlsson, E. & Thesleff, S. *Eur J Biochem* 68, 457–469, 1976.
 Hodgson, W.C., Dal Belo, C.A. & Rowan, E.G. *Neuropharmacol.*, 52, 1229-1236, 2007.
 Hodgson, W.C. & Wickramaratna, J.C. *Clin. Exp. Pharmacol. Physiol.*, 29, 807-814, 2002.
 Su, M.J., Coulter, A.R., Sutherland, S.K. & Chang, C.C. *Toxicon* 21, 143–151, 1983.

NEW TOXIN CLASSES FROM THE VENOMS OF ANGUIMORPHA LIZARDS

Supervisor: A/Prof Wayne Hodgson

Co-supervisor: Dr Bryan Fry

Biochemistry & Molecular Biology, University of Melbourne)



(Dept of

previously
identified

It has only recently been discovered that more lizards are venomous than thought (i.e. >100 instead of only 2). Further, the venoms of these newly species contain previously unidentified toxin classes. This project will involve an examination of two new toxin classes. These toxins are both 'first of type' being completely novel in structure and function. Both toxin types (see below) have been shown to be potently hypotensive but their specific sites/modes of action remain to be elucidated. Techniques to examine these toxins will include a range of *in vitro* (e.g. isolated blood vessels) and *in vivo* (i.e. anaesthetised rats) pharmacological preparations and antagonists/inhibitors.

Toxin type I from the venom of the Haitian Galliwasp (*Celestus warreni*)



Toxin type II from the venom of the Kimberly Rock Monitor (*Varanus glauerti*)



Background reading:

Fry et al (2009) A central role for venom in predation by *Varanus komodoensis* (Komodo Dragon) and the extinct giant *Varanus (Megalania) prisca*. *Proceedings of the National Academy of Science* 106:8969-8974.

Fry et al (2006) Early evolution of the venom system in lizards and snakes. *Nature* 439:509-632.

EXPLORING THE VASOPROTECTIVE ACTIONS OF NITROXYL (HNO)

Supervisors: Dr Alyson A. Miller & Dr Barbara Kemp-Harper

Nitric oxide (NO^{*}), is an important endogenous vasodilator and regulator of vascular tone. In disease states such as stroke, hypertension and atherosclerosis, the NO signaling pathway is impaired leading to reduced blood flow to vital organs such as the brain and heart. However, NO can also exist in the reduced state as nitroxyl (HNO) and HNO donors display unique pharmacological characteristics and have advantages over NO donors in the treatment of cardiovascular diseases such as heart failure. We have evidence to suggest that HNO may also decrease oxidative stress and as such this redox form of NO may represent a new therapeutic target for the treatment of vascular diseases.

The aim of this honours project is to investigate the ability of HNO to limit oxidative stress and serve as protective agent in the cerebral vasculature. It is anticipated that this will involve the use of assays to detect reactive oxygen species generation, small vessel myographs and western blotting in order to examine the function of carotid and cerebral arteries from mice. This study will elucidate the potential vasoprotective effects of HNO and may lead to the development of more effective therapies for vascular disease.

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Role of G-Protein Oestrogen Receptor in the Brain Following Stroke

Supervisors: Dr Brad Broughton and A/Prof Christopher Sobey

Vascular and Pharmacology Immunobiology Group

Stroke is a debilitating disease that can cause permanent neurological damage, complications, and death. At present, there are very few treatment options available for patients and thus, the development of new treatments is vital to reduce the damage caused by stroke. In recent years, growing evidence has revealed that oestrogen plays a protective role in the brain following cerebral ischaemia. For many years it was widely considered that the effects of estrogen were solely attributed to the activation of classical nuclear receptors. However, recent studies have discovered a novel G-protein coupled oestrogen receptor (GPER) in the brain. GPER is highly expressed and extensively distributed throughout the brain, however, it remains to be elucidated as to whether this novel estrogen receptor plays a neuroprotective following stroke.

The aim of this Honours project is to investigate what role GPER plays in the brain of male and female mice following cerebral ischaemia. Techniques that will be used during this project include treating mice with various GPER ligands, histochemical approaches to measure cerebral infarct, immunohistochemistry to examine the distribution of GPER in brain sections and Western blotting to determine GPER expression levels. The scientific knowledge gained during this project will further our understanding of GPER signalling in the brain and potentially lead to new therapeutic strategies to help minimize stroke damage.

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Investigating the role of NADPH oxidase in cerebral artery dysfunction following stroke

Supervisors: Dr Alyson A. Miller & A/Prof Christopher Sobey

Factors regulating blood flow to the brain are numerous and complex. Under normal conditions, brain blood flow is maintained at the appropriate level through fine adjustments to the diameter of the supplying arteries. A “stroke” occurs when a portion of brain tissue is deprived of blood due to blockage or rupture of a cerebral artery, resulting in death of the most severely affected brain cells. Advances in our understanding of abnormal regulation of cerebral arteries in stroke is likely to lead to better treatments to minimize brain damage caused by strokes, or even to prevent strokes from occurring.

The function and integrity of cerebral arteries are critical to support blood flow to the brain following cerebral ischaemia. Increased production of reactive oxygen species (ROS), also known as “oxygen-derived free radicals”, has been linked with cerebral vascular dysfunction following stroke. We have identified that NADPH oxidases are a major source of ROS in the cerebral circulation. However, it is unclear whether NADPH oxidases contribute to ROS levels and subsequent cerebral artery dysfunction following stroke.

The aims of this honours project are; 1) determine whether cerebral ischaemia has a detrimental effect (e.g. impaired endothelial function) on cerebral vascular function following stroke, and 2) elucidate the role of NADPH oxidase in any such changes. It is anticipated that this project will involve the use small vessel myography to assess vascular function, chemiluminescence based assays to measure reactive oxygen species production, and molecular and immunohistochemical approaches to assess protein expression and localisation. This study will provide novel insight into the dysregulation of cerebral artery function after stroke.

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AT₄R/IRAP as a target for protection in cardiovascular disease?

Dr Tracey Gaspari & Dr Robert Widdop

In Australia the largest cause of death is coronary heart disease (CHD) leading to heart attacks or stroke and claiming approximately 30,000 lives a year. The renin angiotensin system (RAS) is well known to participate in the pathogenesis of cardiovascular diseases with overactivity of Ang II instrumental via its effects mediated by the AT₁ receptor. Clinically both angiotensin-converting enzyme (ACE) inhibitors and AT₁ receptor antagonists are effective in preventing events that are associated with atherosclerosis or vascular dysfunction. However, the role of non-AT₁ receptors (AT₂, AT₄ and *Mas* receptor) in the cardiovascular system is not well established.

Currently the evidence supports a role for effects mediated by non-AT₁ receptors to oppose those mediated by the AT₁ receptor in the setting of atherosclerosis. We have recently shown that one of the shorter angiotensin peptides, Ang IV can bind to the AT₄ receptor and provide protection against atherosclerosis. It is now known that the AT₄ receptor is the enzyme, insulin regulated aminopeptidase (IRAP) and that Ang IV could be acting to inhibit the activity of this enzyme.

In collaboration with Dr Siew Yeen Chai at the Howard Florey Institute we plan to use mice that are deficient in the enzyme IRAP to ascertain the exact importance of this enzyme in protection against vascular disease. Therefore the aim of this project will be to investigate the role of IRAP-deficiency in various models of vascular pathology, including models of cardiovascular stress such as Ang II infusion and aortic banding. It is anticipated that the project will involve:

- Surgical procedures
- Morphological analysis of tissue sections
- Immunohistochemistry of tissue sections

This knowledge will aid in the identification of IRAP as a possible important therapeutic target in cardiovascular disease as well as delineating important molecular mechanisms involved in the protective effects mediated by Ang IV.

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Cardiovascular effects of novel AT₂ receptor ligands

Dr Emma Jones & Assoc Prof Robert Widdop

The main effector hormone of the renin angiotensin system (RAS) is angiotensin II which can stimulate both angiotensin AT₁ receptors (AT₁R) and AT₂ receptors (AT₂R). There is evidence to suggest that there is cross talk between AT₁R and AT₂R at both functional and signaling levels.

For example, AT₁R activation is responsible for the classical effects of Ang II such as vasoconstriction, whereas AT₂R activation opposes this effect by direct AT₂R-mediated vasorelaxation or inhibition of AT₁R-mediated signaling.

There is currently intense interest focusing on the AT₂R cardiovascular function, although there are few selective AT₂R ligands available to delineate such effects. *In collaboration with Prof Aguilar (Dept Biochemistry & Molecular Biology), we have exciting preliminary data indicating that a series of novel angiotensin peptide analogues exhibit both in vitro and in vivo vasodilator activity.*

Therefore, the current project will continue this work and will involve biochemical and *in vitro* and *in vivo* cardiovascular experiments identifying lead compounds with AT₂R agonist or antagonist activity. It is anticipated that this project will involve some animal work.

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A ROLE FOR NITROXYL (HNO) IN THE TREATMENT OF NITRATE TOLERANCE IN HUMAN BLOOD VESSELS?

Supervisors: Dr Karen Andrews, Dr Barbara Kemp-Harper & Prof Jaye Chin-Dusting

Arteries play an important role in maintaining blood pressure and supplying the body with the essential nutrients and oxygen for everyday function. All blood vessels have a lining (endothelium) and an outer layer made up of vascular smooth muscle cells. The endothelium releases and produces compounds that act on the smooth muscle layer to control blood pressure and flow by contracting or relaxing (vascular tone). Nitric oxide (NO) is an important and potent compound produced by the endothelium which acts on the smooth muscle layer allowing blood vessels to dilate.

Several conditions and diseases such as hypertension, angina and coronary artery disease exhibit reduced NO production which contributes to abnormal endothelial (vascular) function and reduced blood flow. Treatment of impaired endothelial function includes administration of a drug called GTN (glyceryl trinitrate). GTN, an organic nitrate, releases NO and increases its availability in the blood vessel and induces relaxation. However, a limitation with this drug is the development of nitrate tolerance (the effects of the drug are diminished) that occurs after long-term use.

Recent studies have suggested that nitroxyl (HNO), the reduced congener of NO[•], may be resistant to the development of tolerance. However, the effectiveness of HNO donors in human vessels is not known. Therefore, this project aims to assess the mechanisms of HNO mediated relaxation and its susceptibility to tolerance to determine if HNO has the potential to be used therapeutically. Human saphenous veins and coronary and radial arteries will be obtained from patients undergoing coronary artery graft surgery and mounted in organ baths to assess blood vessel function. Additional techniques include western blot analysis and oxidative stress assays.

PROJECT TITLE: **NOVEL ACTIVATORS OF SOLUBLE GUANYLATE CYCLASE AS NEW TREATMENTS FOR CARDIAC HYPERTROPHY AND FIBROSIS**

Supervisor: Dr Rebecca Ritchie, Dr Jennifer Irvine (Baker IDI Heart & Diabetes Institute, Prahran)
Dr Barbara Kemp-Harper (Dept of Pharmacology, Monash University)

PROJECT DETAILS:

Cardiac hypertrophy, or enlargement of the heart, develops in response to high blood pressure, and is a contributing factor to congestive heart failure. Development of cardiac hypertrophy is thought to result from increased production of oxygen species (ROS) such as superoxide, and activation of growth signals such as mitogen-activated protein kinases (MAPK) and calcineurin. Cardiac fibrosis often accompanies hypertrophy, and together these structural changes can impair myocardial function, such as that seen in heart failure.

We have previously shown the NO•/sGC/cGMP signalling system to act as a powerful cardiac antihypertrophic mechanism. Nitroxyl (HNO) is a novel redox sibling of NO• which may have therapeutic advantages over NO• in the treatment of various cardiovascular diseases. We have recently shown that HNO prevents hypertrophy in isolated cardiomyocytes (heart muscle cells). Moreover, HNO prevents excess generation of superoxide. Yet, its mechanism of action beyond this step has not been elucidated. In addition, we have access to two direct and NO•-independent activators of sGC. These compounds are also resistant to superoxide scavenging and their impact upon the hypertrophic response (including activity of pro-hypertrophic signalling, hypertrophic gene expression) is not yet determined. The impact of the NO•/sGC/cGMP signalling system on cardiac fibrosis is less well studied, but we hypothesise that HNO/sGC ligands also inhibit cardiac fibrosis.

This project, to be performed in **Dr Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran** aims to investigate the mechanisms via which HNO mediates its antihypertrophic action under both physiological and pathophysiological conditions, such as hyperglycaemia, and to compare its effects to that of the NO• donor, DEA/NO and the NO-independent activators of sGC. The scope of this project may include *in vitro* techniques (using cardiomyocytes and/or cardiac fibroblasts), or *ex vivo* (Langendorff-perfused isolated rodent hearts from diabetic vs non-diabetic animals) models of cardiac hypertrophy. This project can be tailored according to the student's abilities and interests and will provide the opportunity for learning a range of techniques, including cell culture, biochemical (Western blot analyses), molecular (real time PCR), physiological and pharmacological techniques.

This study will elucidate the therapeutic potential of HNO and sGC ligands to provide innovative pharmacotherapy for the treatment of heart failure and other cardiovascular diseases.

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PROJECT TITLE: **REACTIVE OXYGEN SPECIES (ROS) INDUCE DAMAGE IN THE DIABETIC HEART: MECHANISMS, CONSEQUENCES AND PREVENTION**

Supervisor: Dr Rebecca Ritchie, Dr Marissa Bowden (Baker IDI Heart & Diabetes Institute, Prahran)
Dr Barbara Kemp-Harper (Dept of Pharmacology, Monash University)

PROJECT DETAILS:

More than one million Australians currently have diabetes, and the disorder is the sixth leading cause of death in our nation, mostly due to cardiovascular disease. Diabetes mellitus and cardiovascular disease are often associated with cardiac fibrosis, cardiomyocyte hypertrophy (enlargement of the heart muscle cells) and a specific dysfunction known as cardiomyopathy. Human diabetic cardiac disease is particularly characterised by early diastolic dysfunction (impaired cardiac relaxation) and late systolic impairment (impaired contractile function). Such impairments may be accompanied with an increased susceptibility to myocardial damage following infarction. This cardiomyopathy, evident in both type 1 and type 2 diabetes, is a major contributor to high morbidity and mortality in diabetic patients. Treatment of the cardiac complications in diabetic patients is still not being adequately achieved, and new interventions specifically for managing these cardiac complications of diabetes are essential.

Recent studies from our lab have shown increased levels of toxic chemicals called reactive oxygen species (ROS) in diabetic heart tissues. ROS are normally removed by naturally-occurring antioxidants in the body, however, diabetic patients have lower amounts of antioxidants in their hearts and in their blood. The elevated levels of ROS in the diabetic heart, along with general decreased antioxidant function, plays a vital role in impairing the heart's ability to pump and relax with each heartbeat, as well as structural changes of the diabetic heart.

This project, to be performed in **Dr Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran** aims to investigate what the downstream mediators of ROS are in the diabetic heart, in terms of changes to detrimental cell signaling, calcium handling proteins and gene expression, and whether we can prevent diabetes-induced myocardial dysfunction and structural changes by upregulating cardioprotective signals in the heart. Depending on the student's abilities and interests, this project will be tailored to use rodent models of type 1 or type 2 diabetes, and the project will provide the opportunity for learning a range of techniques, including *in vitro* techniques (using cardiomyocytes), *ex vivo* (isolated hearts) or *in vivo* approaches. Assessment of myocardial function, as well as histochemical, biochemical (eg Western blot analyses, ROS detection), molecular (real time PCR), physiological and pharmacological techniques will be used.

It is likely that stronger antioxidant approaches and/or drugs that activate protective protein pathways alone or on top of current therapy, will be more effective in treating and thereby reversing, the impaired function and preventing or delaying heart failure, in the diabetic heart.

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