



**DEPARTMENT OF PHARMACOLOGY**  
**HONOURS PROJECTS 2012 (at September 29 2011)**

<b>Labs/ Supervisor(s)</b>	<b>Project Title: ON CAMPUS PROJECTS</b>
<b>Venoms &amp; Toxins Labs:</b> Hodgson/Fry  Konstantakopoulos/ Hodgson/Isbister	<ul style="list-style-type: none"> <li>• Molecular toxinology of Australia's lesser known venomous snakes</li> <li>• Profiling Australian and Malaysian snake venoms to guide treatment strategies.</li> </ul>
<b>VascularBiology &amp; Immunopharmacology Labs:</b> Drummond//Sobey/Lim  Kemp-Harper/Drummond  Chrissobolis/Sobey  Miller/Sobey  Miller/Sobey/Andrews (Physiol)  Selemidis/ Williams (MIMR) et al  Sobey/Jones	<p>Cell based therapies in the treatment of high blood pressure</p> <p>Exploring novel therapeutic strategies to limit ER stress and prevent atherosclerotic plaque rupture</p> <ul style="list-style-type: none"> <li>• Aldosterone and angiotensin II in cerebrovascular disease and stroke</li> <li>• Investigation of Mechanisms Linking Cardiovascular Disease and Alzheimer's Disease</li> <li>• Investigating the Cerebral Vascular Effects of Ghrelin in Hypertension</li> <li>• NADPH oxidase as a regulator of immune suppressor cell function in cancer</li> <li>• AT<sub>2</sub> Receptors in cerebrovascular disease and stroke</li> </ul>
<b>Integrative Cardiovascular Pharmacology Labs:</b> Gaspari/Chai (Physiol)/Widdop  Gaspari/Widdop  Jones/Aguiar (Biochem)/Widdop  Vinh/Widdop	<ul style="list-style-type: none"> <li>• Protective role of AT<sub>4</sub>R/IRAP in cardiovascular disease</li> <li>• Cardioprotective effect of incretin hormones: potential beyond glycaemic control</li> <li>• Drug discovery program for AT<sub>2</sub> receptor ligands</li> <li>• Hypertension Down to the T? The Role of T cells in Hypertension</li> </ul>
<b>Fibrosis Laboratory (new)</b> Chrisan Samuel	<ul style="list-style-type: none"> <li>• Relaxin signal transduction studies</li> <li>• Relaxin efficacy studies</li> <li>• The influence of ageing and gender on fibrosis</li> </ul>
<b>Neuropharmacology</b> Loiacono/Chai (Physiol)	<ul style="list-style-type: none"> <li>• IRAP inhibitors in learning and cognition</li> <li>• Role of IRAP in neurogenesis</li> </ul>
<b>Education-based projects</b> Patak/Hodgson/Davis	<ul style="list-style-type: none"> <li>• Evaluating novel interactive teaching methods (TBA)</li> </ul>
<b>Cardiac Toxicology (Clayton) &amp; VIFM (Sth Melbourne)</b> Andis Graudins	<ul style="list-style-type: none"> <li>• In-vivo model of oral drug poisoning treated with intravenous lipid emulsion: Assessment of cardiovascular and pharmacokinetic outcomes in severe drug poisoning</li> </ul>

<p><b>Drug Discovery Biology Labs: (Monash, Parkville)</b></p> <p><b>Theme leader:</b> Nigel Bunnett (<b>new</b>)</p> <p><b>Theme Leaders:</b> Patrick Sexton, Arthur Christopoulos</p> <p>May/Christopoulos</p> <p>May/Valant</p> <p>Valant/May Leach/ Christopoulos Lane/Christopoulos</p> <p>Lane/Christopoulos</p> <p>Canals/Christopoulos Furness/Wootten/Graham/Sexton Furness/Wootten/Christopoulos/ Sexton Wootten/Sexton</p>	<p><b>OFF CAMPUS PROJECTS</b></p> <ul style="list-style-type: none"> <li>• Bile Acids and Their Receptors: New Mechanisms of Steroid Signalling in the Nervous System</li> <li>• Endosomes: A Legitimate Platform for the Signalling Train</li> <li>• Proteases: Gnawing Away and Inflammation and Pain</li> </ul> <ul style="list-style-type: none"> <li>• Structural basis of allosteric modulator interactions at adenosine A<sub>1</sub> receptors</li> <li>• Understanding adenosine receptor signalling under hypoxic conditions</li> <li>• The effects of pH on adenosine A<sub>1</sub> receptor pharmacology</li> <li>• Mutational Analysis of the Calcium Sensing Receptor</li> <li>• Molecular mechanisms of a novel allosteric modulator of the D<sub>2</sub>-like dopamine receptors</li> <li>• Stimulus bias at the dopamine D<sub>2</sub> receptor and its role in the treatment of schizophrenia</li> <li>• Ligand-biased signalling of the <math>\delta</math> opioid receptor</li> <li>• Signalling bias of Calcitonin receptor polymorphs</li> <li>• Biochemical pharmacology of GPCR activation states</li> </ul> <ul style="list-style-type: none"> <li>• Elucidation of allosteric binding sites and structural determinants of allosteric agonism and modulation at the glucagon-like 1 peptide receptor</li> </ul>
<p><b>BakerIDI (Prahran) (Chin-Dusting Lab)</b> Andrews/Chin-Dusting/Kemp-Harper Sampson/Irvine/Chin-Dusting/ Widdop</p>	<ul style="list-style-type: none"> <li>• Role for nitroxyl in the treatment of inflammation</li> <li>• Potential anti-inflammatory actions of AT<sub>2</sub> receptor ligands in vasculature</li> <li>•</li> </ul>
<p><b>BakerIDI (Prahran) (Ritchie Lab)</b> Ritchie/Kemp-Harper</p>	<ul style="list-style-type: none"> <li>• Nitroxyl, a relative of NO, is a naturally-occurring cardioprotective molecule</li> <li>• New strategies to rescue diabetes-induced cardiac dysfunction</li> <li>• Targeting the anti-inflammatory protein Annexin-A1 for protection from myocardial infarction (heart attack)</li> </ul>
<p><b>BakerIDI (Prahran) (Head Lab)</b> Geoff Head/ Pamela Davern</p>	<ul style="list-style-type: none"> <li>• Role of neuropeptides in the amygdala mediating neurogenic hypertension</li> <li>• Neurochemical subtype of stress responsive neurons leading to hypertension</li> <li>• Neurogenic hypertension in female Schlager hypertensive mice</li> <li>• Role of brain pathways in chronic stress</li> </ul>

**For more details (Hons Convenor):**  
**robert.widdop@monash.edu**  
**9905 4858**

## HONOURS PROJECT 2011

### Solving the “Brown snake paradox” (part 2)

Supervisor: Professor Wayne Hodgson

Co-supervisors: A/Professor Geoff Isbister (Newcastle) / Dr Richard Loiacono

The Eastern Brown Snake (*Pseudonaja textilis*) is one of the world’s most venomous snakes with a murine LD<sub>50</sub> 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).

**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)

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 hypothesized that the absence of neurotoxicity in Brown snake bites is due to only small amounts of the potent textilotoxin being present in the venom. We have recently confirmed this hypothesis and also shown that the amount of textilotoxin in the venom of the Brown snake was markedly lower than the amount of taipoxin in the venom of the taipan (i.e. approx 5% versus 20%). While this data partially explains the lack of neurotoxicity in humans envenomed by the Brown snake it doesn’t explain why the post-synaptic toxins in the venom of the Brown snake are ‘in active’ in humans. This study will isolate and then compare the neurotoxicity of post-synaptic toxins venom from brown snake (*P. textilis*) with those from the taipan (*O. scutellatus*) using skeletal muscle preparations and a human cell line containing nicotinic receptors. The post-synaptic neurotoxins will be isolated from the venoms using reverse-phase HPLC.

The aim is to determine if there is a difference in the ability of post-synaptic neurotoxins to inhibit (or bind to) nicotinic receptors from humans in comparison to those from avian and other mammalian preparations in an attempt to explain the lack of neurotoxicity in envenomed patients.

### References

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 Su, M.J., Coulter, A.R., Sutherland, S.K. & Chang, C.C. *Toxicon* 21, 143–151, 1983.

## HONOURS PROJECT 2011

### Molecular toxinology of Australia's lesser known venomous snakes

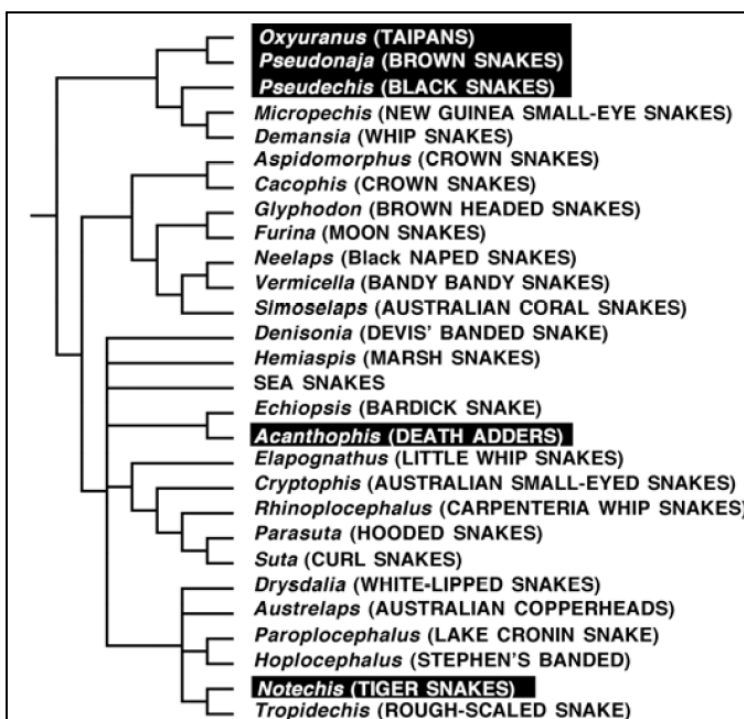
Supervisor: Professor Wayne Hodgson

Co-supervisor: Dr Bryan Fry (University of Melbourne)

Australia is home to a remarkable number of extremely venomous snakes. Despite the inherent medical importance and bioprospecting importance, the vast majority of the species remain poorly studied. This project will examine for the first time the pharmacological/biochemical activity of these unique venoms using multiple techniques. This will result in better understanding of the fundamental nature of the venoms, leading to disparate outcomes including evolutionary interpretation, potential lead compounds for use in drug design and development, and even better management of envenomed patients.

#### The aims of the study are to:

- Characterise the molecular biodiversity of mutated proteins/peptides within the venoms of unstudied, potentially medically important Australian snakes.
- Characterise the bioactivities of the crude venoms and purified toxins
- Explore novel small venom peptides as potential lead candidates for drug design and development



**Figure 1:** Genus-level taxonomical relationships of Australian venomous snakes. Highly studied terrestrial species are highlighted.

Techniques will include HPLC, MALDI-TOF, coagulation studies, in vitro and in vivo preparations.

## ***Profiling Australian and Malaysian snake venoms to guide treatment strategies.***

Supervisors:

Dr Nicki Konstantakopoulos, Professor Wayne Hodgson, A.Professor Geoff Isbister

Australia is host to a vast array of venomous terrestrial and aquatic snakes belonging to the Elapidae family. The snake venoms' are comprised of a multitude of pharmacologically active components used for the capture of prey. Clinical manifestations presented following envenomation may comprise of one or more manifestations, including neurotoxic symptoms, haemolytic or necrotic. Not all snake envenomation, however, results in the same effects. This may be due to variation in venom composition due to species variation, location of habitat, age or gender.



Commercially available antivenom manufactured in Australia by the Commonwealth Serum Laboratories (CSL) are commonly used to treat envenomed patients. The antibodies are isolated from horses inoculated with snake venom. Whilst used successfully, like many medications they pose potential side-effects. The most concerning of the side effects is anaphylactic shock, most likely to be displayed by patients previously exposed to equine antibodies.

This study will determine the cytotoxicity, pro-coagulation and neurotoxic activity of two Australian elapids and one Malaysian elapid. Cytotoxicity will be determined using a cell based assay to determine the inhibitory concentration (IC) of cellular proliferation on rat skeletal muscle cells, using an established rat skeletal cell line, L6. Coagulation studies will be performed by incubating venom with human blood plasma (obtained from Red Cross Blood Bank) and measuring the length of time it takes for the blood plasma to clot. Neurotoxicity will be determined using chick biventer cervicis nerve-muscle preparations to determine the effects of venom in twitch response.

Antibodies raised against the three snake species, as well as commercially available antivenom will also be co-administered along with venom to determine their ability to inhibit the effects caused by the venom.

### **Contacts:**

Dr Nicki Konstantakopoulos

Email: [nicki.konstantakopoulos@monash.edu](mailto:nicki.konstantakopoulos@monash.edu)

Tel: 9905 0913

Professor Wayne Hodgson

Email: [wayne.hodgson@monash.edu](mailto:wayne.hodgson@monash.edu)

Tel: 9905 4861

## CELL-BASED THERAPIES IN THE TREATMENT OF HIGH BLOOD PRESSURE

Hypertension (high blood pressure) is a major cause of heart failure, heart attacks and strokes. Over 2 million people in Australia suffer from hypertension and alarmingly, in up to 30% of these individuals, the condition is not controlled by current blood pressure medications. A recent development in the field of hypertension research is the recognition that the disease is caused by an influx of immune cells (i.e. T cells and macrophages) into the artery wall. Subsequent activation of these immune cells leads to the release of free radicals and cytokines that promote an inflammatory response, causing the arteries to become 'stiff' and constricted and exacerbating elevations in blood pressure. Hence, strategies that can reduce inflammation in the vascular wall hold promise as future treatments for hypertension.

Stem cells have long been touted as possible cures for degenerative diseases on the basis of their tissue regenerative properties. However, stem cells also display powerful anti-inflammatory properties suggesting that they may also have therapeutic potential in inflammatory conditions such as hypertension. The human amniotic membrane is a readily available tissue (discarded after childbirth) and a rich source of stem cells. Furthermore, stem cells derived from the human amnion are superior in many ways to stem cells derived from other tissues (e.g. embryos, bone marrow) in being immunologically inert, having little or no potential for tumour generation, and also displaying particularly powerful anti-inflammatory properties.

The current project will involve *in vivo* studies in mice to test the hypothesis that ***human amnion stem cells prevent hypertension by exerting powerful anti-inflammatory effects in the artery wall and in other organs that regulate blood pressure, including the kidney and brain.*** By taking on this project, the student will have the opportunity to learn a range of scientific techniques including minor surgical procedures and conscious blood pressure monitoring in mice, FACS analysis to identify stem cells and immune cells in mouse tissues, molecular biology including real-time PCR and Western blotting, and functional studies on isolated blood vessels.

For further information please contact:

**Dr Grant Drummond**

Phone: 9905 4869

[Grant.Drummond@monash.edu](mailto:Grant.Drummond@monash.edu)

**A/Prof Chris Sobey**

Phone: 9905 4189

[Chris.Sobey@monash.edu](mailto:Chris.Sobey@monash.edu)

**Dr Rebecca Lim**

Phone: 9902 4775

[Rebecca.Lim@monash.edu](mailto:Rebecca.Lim@monash.edu)

# EXPLORING NOVEL THERAPEUTIC STRATEGIES TO LIMIT ER STRESS AND PREVENT ATHEROSCLEROTIC PLAQUE RUPTURE

Supervisors: Dr Barbara Kemp-Harper & Dr Grant Drummond

Atherosclerosis is characterised by the formation of lipid filled lesions in the arterial wall. These plaques are relatively harmless until they become unstable and rupture leading to thrombus formation and vessel occlusion resulting in myocardial infarction and stroke. Recent evidence suggests that dysfunction of the endoplasmic reticulum (ER), which is involved in protein synthesis and folding, leads to oxidative stress and cell death and plaque destabilisation. This ER stress response is evident in macrophages and endothelial cells in atherosclerotic lesions and limiting ER stress may represent a novel strategy to stabilise atherosclerotic plaques. We have evidence to suggest that nitroxyl (HNO), a novel redox form of nitric oxide, and NADPH oxidase inhibitors may decrease ER stress and stabilize atherosclerotic plaques.

The aim of this honours project is to investigate the ability of HNO and NADPH oxidase inhibitors to limit ER stress and serve as protective agents against atherosclerotic plaque rupture. It is anticipated that this will involve the use of assays to detect ER stress (western blotting, immunohistochemistry), reactive oxygen species generation (chemiluminescence) and plaque formation and stability in atherosclerotic mice. This study will elucidate the potential vasoprotective effects of HNO and NADPH oxidase inhibitors and may lead to the development of more effective therapies for the treatment of atherosclerosis.

**Dr Barbara Kemp-Harper and Dr Grant Drummond**

Department of Pharmacology

Monash University

Phone: 9905 4674, Rm E140

[Barbara.Kemp@monash.edu](mailto:Barbara.Kemp@monash.edu)

[Grant.Drummond@monash.edu](mailto:Grant.Drummond@monash.edu)

## **Aldosterone and angiotensin II in cerebrovascular disease and stroke**

**Supervisors: Dr Sophocles Chrissobolis & A/Prof Christopher Sobey**

*Vascular Biology and Immunopharmacology Group*

Stroke is the 2nd leading cause of death in Australia, thus it is of the highest priority to better understand the complex contributing mechanisms in order to more effectively treat it. This project will test whether increased levels of aldosterone and angiotensin II (Ang II) are critical components of mechanisms contributing to worsened stroke outcome (even in the absence of hypertension). We will also test whether aldosterone or Ang II increases oxidative stress and impairs endothelial function in the cerebral circulation, and augments apoptotic signalling and cell death in an in vitro model of cerebral ischaemia. We will determine if mineralocorticoid receptors or G-protein coupled receptor 30 are involved in any of the effects of aldosterone or Ang II. The proposed studies will use pharmacological and genetic approaches and both in vitro and in vivo models of cerebral ischaemia.

We predict that subpressor levels of aldosterone and angiotensin II contribute to cerebrovascular disease and worse outcome after stroke, even when anti-hypertensive drugs are used effectively to eliminate clinical hypertension.

Dr Sophocles Chrissobolis (Rm 139)

Ph: 9905 0914

[Sophocles.Chrissobolis@monash.edu](mailto:Sophocles.Chrissobolis@monash.edu)

A/Prof Christopher Sobey (Rm 148)

Ph: 9905 4189

[chris.sobey@monash.edu](mailto:chris.sobey@monash.edu)

## **Investigation of Mechanisms Linking Cardiovascular Disease and Alzheimer's Disease**

**Supervisors: Dr Alyson Miller and A/Prof Christopher Sobey**  
**Vascular Biology and Immunobiology Group**

Traditionally, Alzheimer's disease has, by definition, been categorized as a 'non-vascular' neurodegenerative dementia. However, it is now widely recognised that most dementias and almost half of clinically diagnosed Alzheimer's patients have cerebral blood vessel abnormalities. These observations, in concert with epidemiological studies indicating that Alzheimer's disease and cerebrovascular disease share the same risk factors (e.g. hypertension, hypercholesterolemia), supports the novel concept that vascular factors play a prominent role in the initiation and development of Alzheimer's disease.

The aim of this Honours project is to firstly demonstrate that cardiovascular risk factors accelerate the onset and progression of Alzheimer's-like molecular and cognitive changes. Secondly, this project will explore potential pathways and mediators that mechanistically link cardiovascular risk factors and Alzheimer's disease, with a particular focus on oxidative and inflammatory processes. This project will utilize clinically relevant mouse models of cardiovascular and Alzheimer's disease. Techniques that will be used during this project include small vessel myography to assess vascular function, chemiluminescence based assays to assess oxidative stress, and molecular approaches and flow cytometry to assess inflammation. It is anticipated that this project will significantly advance our understanding of how vascular factors influence Alzheimer's disease pathology, and may lead to the identification of novel therapeutic targets to maintain brain health and delay cognition in patients with neurodegenerative and cardiovascular diseases.

**Dr Alyson A. Miller**  
**Department of Pharmacology**  
**Phone: 9905 3817**  
**E-mail: [Alyson.Miller@monash.edu](mailto:Alyson.Miller@monash.edu)**

**A/Prof Christopher Sobey**  
**Department of Pharmacology**  
**Phone: 9905 4189**  
**E-mail: [Chris.Sobey@monash.edu](mailto:Chris.Sobey@monash.edu)**

## **Investigating the Cerebral Vascular Effects of Ghrelin in Hypertension**

**Supervisors: Dr Alyson Miller, A/Prof Christopher Sobey & Dr Zane Andrews**  
**Vascular Biology and Immunobiology Group & Neuronal and Degenerative Laboratory**

The brain lacks sufficient energy reserves and is critically dependent on a continuous and well-regulated blood supply to support its dynamic needs for oxygen and glucose. Complex regulatory mechanisms assure that the brain receives sufficient blood flow to maintain its functions. Hypertension disrupts these regulatory mechanisms and increases the susceptibility of the brain to stroke and cognitive impairment. A large and expanding body of evidence indicates that oxidative stress and inflammation play prominent roles in the vascular abnormalities caused by hypertension. Thus, identification of pathways and mediators that protect against such mechanisms is likely to lead to better treatments to lower stroke risk and cognitive decline associated with hypertension.

Ghrelin is a peptide hormone synthesized predominantly in the stomach, where it is secreted into the circulation. Ghrelin is best known for its ability to regulate energy homeostasis and body weight by stimulating growth hormone release from the pituitary gland. Besides its central effects, it is now apparent that ghrelin has a broad range of peripheral functions, including roles in the cardiovascular and immune systems. For example, ghrelin exerts vasodilatory effects, inhibits the production of pro-inflammatory cytokines by immune cells, and may limit oxidative stress in cerebral blood vessels. Taken together, these observations raise the possibility that ghrelin-mediated signaling may represent a novel vasoprotective pathway. Thus, the aim of this Honours project is to investigate the influence of ghrelin on oxidative and inflammatory processes in the cerebral circulation of hypertensive mice. It is anticipated that this project will involve the use small vessel myography to assess cerebral vascular function, chemiluminescence based assays to assess oxidative stress, and molecular approaches and FACS analysis to assess inflammation.

**Dr Alyson A. Miller**  
**Department of Pharmacology**  
**Phone: 9905 3817**  
**E-mail: [Alyson.Miller@monash.edu](mailto:Alyson.Miller@monash.edu)**

**A/Prof Christopher Sobey**  
**Department of Pharmacology**  
**Phone: 9905 4189**  
**E-mail: [Chris.Sobey@monash.edu](mailto:Chris.Sobey@monash.edu)**

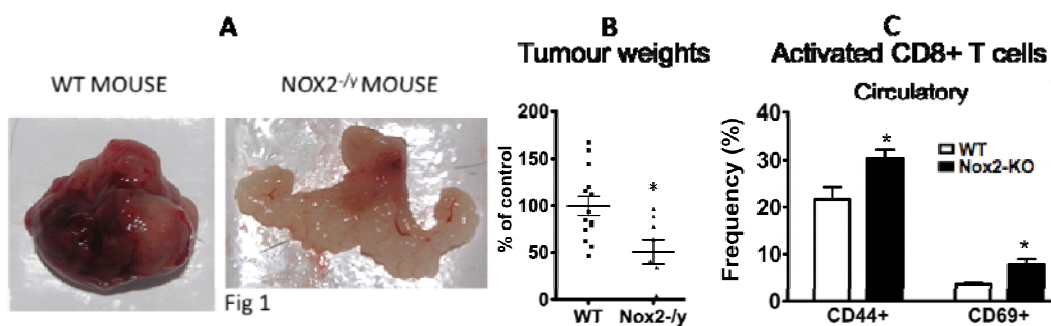
**Dr Zane Andrews**  
**Department of Physiology**  
**Phone: 9905 8165**  
**E-mail: [Zane.Andrews@monash.edu](mailto:Zane.Andrews@monash.edu)**

# NADPH OXIDASE AS A REGULATOR OF IMMUNE SUPPRESSOR CELL FUNCTION IN CANCER

**Project description:** The socio-economic burden of cancer is profound, claiming many lives in Australia and worldwide. A substantial body of evidence indicates that one of the most important hallmarks of cancer is angiogenesis — the process of new blood vessel formation<sup>1,2</sup>. This process allows solid tumours to grow beyond a few millimetres in diameter and then to metastasise and spread to distant sites. Therefore, there has been a keen interest in developing drugs to target angiogenesis for cancer therapy. *Although much effort has been directed towards identification of the genetic and molecular mechanisms that lead to angiogenesis in cancer, current anti-angiogenic drugs fail to prevent revascularization.*

Currently, there are two new paradigms in angiogenesis research. The first defines a central role for chronic inflammation involving immune suppressor cells such as macrophages (CD11b<sup>+</sup>F4/80<sup>+</sup>Foxp3<sup>+</sup>), T regulatory lymphocytes (Tregs; CD25<sup>+</sup>CD4<sup>+</sup>Foxp3<sup>+</sup>) and bone marrow derived suppressor cells (MDSCs; CD11b<sup>+</sup>Gr-1<sup>+</sup>) and the second implicates reactive oxygen species (ROS) production. Suppressor cells dampen the immune system such as CD8<sup>+</sup> T cells and natural killer cells, which are important cells, as they are known to possess tumouricidal activity. It is largely unknown how suppressor cells are regulated and mediate their suppressive effects but recent evidence suggests that ROS, and in particular those generated by the Nox2 isoform of the NADPH oxidase enzyme family (Nox2 oxidase) are key players.

## Key preliminary data from our laboratories:



We have several lines of evidence indicating that Nox2 oxidase-derived ROS promote tumour formation. First, we observed a profound and significant reduction in the ability of prostate cancer cells to form progressive tumours when injected into the

prostate of mice genetically deficient in Nox2 oxidase (i.e. Nox2<sup>-/-</sup> mouse) compared to normal WT mice (Fig 1A and B). Second, H & E staining showed a significant reduction in tumour angiogenesis in the Nox2<sup>-/-</sup> mouse. Third, using flow cytometry, we showed significantly ( $P < 0.05$ ;  $n = 6$ ) increased proportions of circulating- *activated/effector* (i.e. CD69<sup>+</sup> and CD44<sup>+</sup>) CD8<sup>+</sup> T cells in Nox2<sup>-/-</sup> mice (Fig 1C).

*Our novel data and published literature clearly indicate that suppression of Nox2 oxidase markedly suppresses angiogenesis and tumour growth. This may occur as a result of a reduction in the activities of tumour promoting suppressor cells.*

## Techniques to be performed in the Department of Pharmacology

1. Multi-disciplinary approaches (e.g. in vivo animal models, flow cytometry, Nox knockout mice, adoptive transfer, chimeric mice) to characterize the key suppressor cells that infiltrate the tumour and promote its progression.

**Project supervisor: Dr Stavros Selemidis**

(03) 9905-5756 or email on [stavros.selemidis@monash.edu](mailto:stavros.selemidis@monash.edu)

**Collaborators: Dr Elizabeth Williams, Dr Antony Vinh, Dr Grant Drummond, A/Prof Chris Sobey**

## **AT2 Receptors in cerebrovascular disease and stroke**

**Supervisors: A/Prof Chris Sobey and Dr Emma Jones**

Although stroke is the 2<sup>nd</sup> leading cause of death in Australia, we still have no treatments that can be given to a patient to limit mechanisms of ischaemic brain damage and/or promote functional recovery. Many promising experimental drugs for stroke have failed in the clinic for a variety of reasons. The only treatment currently available (tPA) merely breaks down a blood clot that has interrupted brain blood flow, and it can only be given to about 10% of stroke patients. We therefore desperately need to identify new mechanisms and drug classes that might have potential for stroke therapy.

This project will test whether a range of novel agonists of the angiotensin AT2 receptor might have protective effects in experimental stroke models. Studies will utilise both in vitro (cultured neurons, isolated cerebral arteries) and in vivo (mouse model of cerebral ischaemia) to evaluate AT2 receptor agonists and related drugs for their ability to reduce neuronal damage, oxidative stress, apoptosis, and ultimately cognitive function following stroke.

A/Prof Christopher Sobey (Rm 148)

Ph: 9905 4189

[chris.sobey@monash.edu](mailto:chris.sobey@monash.edu)

Dr Emma Jones (Rm 134)

Ph: 9905 9419

[emma.jones@monash.edu](mailto:emma.jones@monash.edu)

# **Integrative Cardiovascular Pharmacology Labs:**

**Dr Tracey Gaspari & Professor Robert Widdop**

## **Research Focus**

Our research focuses on understanding the pathophysiological processes involved in the development of cardiovascular disease, one of the major causes of illness and death in Australia and worldwide. Projects outlined below could incorporate a range of methodologies including animal dietary and pharmacological treatments, surgical techniques for vascular injury models, immunohistochemistry, biochemical measures (western blot, markers of cellular proliferation and hypertrophy), histological and morphological analyses (such as fibrosis, intimal to medial measurements).

### **Project 1: Protective role of AT<sub>4</sub>R/IRAP in cardiovascular disease? (in collaboration with Dr Siew Yeen Chai – Physiology Dept)**

We have recent evidence that angiotensin IV, acting via the AT<sub>4</sub>R, is athero-protective. The AT<sub>4</sub>R has been identified as the enzyme, insulin regulated aminopeptidase (IRAP), with Ang IV postulated to mediate effects by inhibiting the catalytic activity of this enzyme. Thus this project aims to elucidate the importance of inhibiting this enzyme in treating cardiovascular disease. To do this, projects will examine effect of various cardiovascular stressors in IRAP deficient and control mice and elucidate underlying mechanisms that may mediate any protective vascular or cardiac effects.

### **Project 2: Cardioprotective effect of incretin hormones: potential beyond glycaemic control? (in collaboration with Dr Anthony Dear, ACBD, Monash)**

Two classes of diabetic drugs targeting the glucagon-like peptide-1 (GLP-1) pathway are used clinically to treat Type 2 Diabetes Mellitus. These are GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors (prolong ½ life of endogenous GLP-1). In collaboration with Dr Dear we aim to have projects that investigate the vascular and cardio-protective effect of these classes of drugs in a number of cardiovascular disease states, including atherosclerosis, vascular injury (neointimal hyperplasia) and hypertension.

## **Supervisors:**

Dr Tracey Gaspari  
Ext. 54762  
[tracey.gaspari@monash.edu](mailto:tracey.gaspari@monash.edu)

Professor Robert Widdop  
Ext. 54858  
[robert.widdop@monash.edu](mailto:robert.widdop@monash.edu)

## ***Drug discovery program for AT<sub>2</sub> receptor ligands***

*Dr Emma Jones, Prof Mibel Aguilar (Biochem) & Prof Robert Widdop*

The main effector hormone of the renin angiotensin system (RAS) is angiotensin II which can stimulate both angiotensin AT<sub>1</sub> receptors (AT<sub>1</sub>R) and AT<sub>2</sub> receptors (AT<sub>2</sub>R). There is evidence to suggest that there is cross talk between AT<sub>1</sub>R and AT<sub>2</sub>R at both functional and signaling levels. For example, AT<sub>1</sub>R activation is responsible for the classical effects of Ang II such as vasoconstriction, whereas AT<sub>2</sub>R activation opposes this effect by direct AT<sub>2</sub>R-mediated vasorelaxation or inhibition of AT<sub>1</sub>R-mediated signaling. There is currently intense interest focusing on the AT<sub>2</sub>R cardiovascular function, although there are few selective AT<sub>2</sub>R ligands available to delineate such effects.

We have embarked on a drug discovery program replacing natural amino acids in the Ang II molecule with synthetic amino acid derivatives (e.g. beta-substituted amino acids). Our preliminary data have identified a series of novel angiotensin peptide analogues that exhibit AT<sub>2</sub>R selectivity, evidenced from 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 AT<sub>2</sub>R agonist or antagonist activity. This project will involve animal work including:

- Surgical procedures
- *In vitro* studies of vascular reactivity
- *In vivo* measurement of blood pressure
- Cell culture
- Signaling assays

Emma Jones  
Robert Widdop

emma.jones@monash.edu  
robert.widdop@monash.edu

## Hypertension Down to the T? The Role of T cells in Hypertension

*Dr Antony Vinh & Assoc Prof Robert Widdop*

Hypertension is a major risk factor for cardiovascular diseases such as myocardial infarction, stroke and atherosclerosis. Despite extensive research, the etiology of most cases of adult hypertension remains unclear. A growing body of literature has implicated inflammation, and in particular adaptive immunity and T cells, in hypertension. The role of T cells in the genesis of hypertension is currently very topical. Recently it was shown that T cells are required for the development of hypertension since RAG-1-deficient mice that lack T cells, exhibit a blunted response to experimental hypertension. It has been suggested that the development of hypertension involves T cells, activated from hypertensive stimuli, infiltrating target organs such as the vasculature, brain and kidneys, which are vital organs that regulate blood pressure control.

Currently, inhibitors of the renin-angiotensin system (RAS) represent our frontline therapeutics to regulate severe cases of hypertension. However, it is not clearly understood how these drugs affect T cell function, particularly after chronic treatment. Since T cells are known to express components of the RAS, it is feasible that this system may have large implications on their function, which we now know includes blood pressure modulation. Therefore, we hypothesize that the RAS can modulate T cell function, which contributes to the maintenance of blood pressure. This project will address this hypothesis through the following aims:

1. To determine the effect of chronic RAS inhibition on T cell function in hypertension.
2. To characterize circulating T cell phenotypes and extent of infiltration in mice with AT<sub>1</sub>R- and AT<sub>2</sub>R-deficiency in response to hypertension.

Techniques involved in this project:

- Animal handling and surgery
- Mouse blood pressure monitoring
- Flow cytometry
- Histology
- Immunohistochemistry/immunofluorescence

This proposal therefore, aims to investigate the possible link between the RAS and T cell activation and function in the setting of hypertension.

Suggested Reading:

Guzik et al, 2007. *J Exp Med.* 204(10): 2449-60

Harrison et al, 2010. *Curr Opin Pharmacol.* 10(2):203-7

Antony (Bill) Vinh  
Robert Widdop

antony.vinh@monash.edu  
robert.widdop@monash.edu

## HONOURS PROJECTS (2012) – Fibrosis Laboratory

### Team Leader:

Dr Chrishan S. Samuel, PhD (Senior Research Fellow)

### Contact details:

**Email:** [chrishan.samuel@monash.edu](mailto:chrishan.samuel@monash.edu)

**Phone:** +61 3 9902 0152

**Facsimile:** +61 3 9905 2547

### Research Interests:

Fibrosis is defined as the hardening and/or scarring of various organs including the heart and kidney, which usually results from abnormal wound healing to tissue injury, resulting in an excessive deposition of extracellular matrix components, primarily collagen. The eventual replacement of normal tissue with scar tissue leads to organ stiffness and ultimately, organ failure. Despite a number of available treatments for patients with various heart/kidney diseases, patients receiving these therapies still progress to end-stage organ failure due to the inability of these treatments to directly target the build-up of fibrosis. Hence, novel and more direct anti-fibrotic therapies are still required to be established.

The Fibrosis Lab focuses on the anti-fibrotic actions of the naturally occurring, hormone relaxin, which is mainly produced in the ovary of pregnant mammals, the prostate and testes of males as well as a number of non-reproductive organs - and has a number of beneficial effects in the body including an ability to prevent inflammatory reactions, tissue fibrosis, oxidative stress and cell death, vasoconstriction of blood vessels and organ hypertrophy, while promoting angiogenesis and blood vessel growth in addition to stem cell survival. Several studies have shown that relaxin prevents and/or reverse fibrosis in various experimental models of heart and kidney disease (fibrotic cardiomyopathy, hypertension, myocardial infarction, diabetic cardiomyopathy, tubulointerstitial renal fibrosis) regardless of etiology. The hormone achieves its actions by disrupting transforming growth factor (TGF)- $\beta$ 1 activity (the major pro-fibrotic factor that promotes collagen production and scar tissue accumulation), while augmenting matrix metalloproteinase (MMP) activity (which are enzymes that mediate the breakdown of collagen).

However, further work, which will form the basis of potential honours projects requires 1) an investigation of relaxin signal transduction pathways by which it mediates its anti-fibrotic actions – to identify novel therapeutic targets that may be utilised to enhance its anti-scarring actions; and 2) comparing its anti-fibrotic actions to currently available therapies such as ACE inhibitors, angiotensin receptor blockers and aldosterone receptor blockers – to demonstrate its effectiveness as a suitable anti-fibrotic and/or adjunct therapy.

### Available Projects:

#### 1. Relaxin signal transduction studies

We have recently shown that relaxin activation of its G-protein coupled receptor, RXFP1, initiates a sequence of events involving nNOS-NO-cGMP to disrupt Smad2 phosphorylation (a regulatory protein that promotes TGF- $\beta$ 1 activity), and hence, the fibrosis-promoting actions of TGF- $\beta$ 1 (Mookerjee I et al., 2009 FASEB J 23:1219-1229). Using primary human and/or rat fibroblast cultures (collagen producing cells which naturally express RXFP1) and pharmacological inhibitors to various signal transduction pathways – additional pathways by which relaxin signals through to disrupt the actions of TGF- $\beta$ 1 and increase MMP activity will be studied. In particular, the angiotensin II, NADPH oxidase and inflammatory pathways (which are all involved in promoting TGF- $\beta$ 1 activity) will be investigated.

#### 2. Relaxin efficacy studies

Relaxin is a rapidly-occurring but safe anti-fibrotic, that prevents and/or reverses fibrosis in various experimental models of heart and kidney disease (reviewed in Samuel CS et al. 2006 Pharm Therap 112:529-552; Du XJ et al. 2010 Nat Rev Cardiol 7:48-58).

To further demonstrate its effectiveness as a suitable anti-fibrotic and/or adjunct therapy, the actions of relaxin will be compared to or added to currently used therapies for patients with heart/kidney disease (ACE inhibitors, angiotensin receptor blockers or aldosterone receptor blockers) – in models of ischemic heart disease and obstructed kidney disease; which undergo fibrosis progression in the absence of blood pressure changes.

### **3. The influence of ageing and gender on fibrosis**

Male, but not female relaxin knockout mice undergo an age-related progression of cardiac (Du XJ, Samuel CS et al. 2003 *Cardiovasc Res* 57:395-404) and renal (Samuel CS et al. 2004 *Kidney Int* 65:2054-2064) fibrosis; which provides a model to study why men are more prone to heart and kidney disease compared to women.

Using aged and injury-induced relaxin, aromatase and/or androgen receptor knockout mice, the interaction between relaxin, estrogen and testosterone will be further studied to delineate the contributions of each hormone to disease/fibrosis progression.

#### **Laboratory Techniques:**

- Animal surgery/pathophysiology
- Functional studies
- Cell culture
- Protein Biochemistry
- Molecular Biology
- Histology/immunohistochemistry

## **HONS PROJECTS 2012**

**(1) Siew Chai (Dept Physiology, Monash University) / (2) Richard Loiacono (Dept Pharmacology, Monash University)**

### **IRAP INHIBITORS IN LEARNING AND COGNITION**

#### **BACKGROUND**

When injected into the brains of rodents Ang IV improves aspects of memory and cognition. While originally thought to mediate this effect through a receptor for AngIV termed the AT<sub>4</sub> receptor it has since been demonstrated that the target for Ang IV is the metalloproteinase IRAP (insulin regulated aminopeptidase) which is localised within regions of the brain known to be important for memory and cognition, such as the hippocampus. As such IRAP has been implicated in several neuronal processes and as such presents as a novel target for combating cognitive decline. Ang IV (and newer analogs) act as inhibitors of IRAP rather than classical agonists at this site

Several analogues share the behavioural and pharmacological properties of Ang IV, and several new compounds have been synthesised to better target this enzyme, however, the mechanism(s) of these compounds is still unclear.

#### **AIM**

The main aim of this project is to characterise the actions of a new potent IRAP inhibitors in learning and cognition

#### **TECHNIQUES**

Behavioural studies in mice to characterise the effects of IRAP inhibitors on memory and learning  
Cell culture / molecular biochemical studies using neuronal cell types.

#### **ADDITIONAL READING**

AT<sub>4</sub> receptor is insulin-regulated membrane aminopeptidase: potential mechanisms of memory enhancement (2003) Albiston et al., TRENDS in Endocrinology and Metabolism 14, 72-77

## **HONS PROJECTS 2012**

**(1) Richard Loiacono (Dept Pharmacology, Monash University) / (2) Siew Chai (Dept Physiology, Monash University)**

### **ROLE OF IRAP IN NEUROGENESIS**

#### **BACKGROUND**

When injected into the brains of rodents Ang IV improves aspects of memory and cognition. While originally thought to mediate this effect through a receptor for AngIV termed the AT<sub>4</sub> receptor it has since been demonstrated that the target for Ang IV is the metallopeptidase IRAP (insulin regulated aminopeptidase) which is localised within regions of the brain known to be important for memory and cognition, such as the hippocampus. As such IRAP has been implicated in several neuronal processes and as such presents as a novel target for combating cognitive decline. Ang IV (and newer analogs) act as inhibitors of IRAP rather than classical agonists at this site

IRAP KO (knockout) mice would be expected to perform better in memory tasks, however, this was not the case. Contrary to expectation, the IRAP KO mice exhibited deficits in memory. This surprising finding could be due to altered normal brain development as a result of the loss of IRAP.

#### **AIM**

The main aim of this project is to characterise the role of IRAP in neurogenesis and behaviour

#### **TECHNIQUES**

Behavioural studies in IRAP KO mice to characterise the role of IRAP inhibitors on memory and learning. Imaging / molecular biochemical studies to follow the process of neurogenesis in brain development.

#### **ADDITIONAL READING**

AT<sub>4</sub> receptor is insulin-regulated membrane aminopeptidase: potential mechanisms of memory enhancement (2003) Albiston et al., TRENDS in Endocrinology and Metabolism 14, 72-77

**In-vivo model of oral drug poisoning treated with intravenous lipid emulsion: Assessment of cardiovascular and pharmacokinetic outcomes in severe drug poisoning.**

**Contact: Professor Andis Graudins**

Intravenous lipid emulsion (ILE) has been touted as an antidote to lipid soluble drugs producing severe CVS instability in overdose. In-vitro evidence has shown that serum drug concentrations are reduced in the presence of ILE, suggesting a potential 'lipid-sink' phenomenon in the presence of the lipid emulsion. Several animal models have assessed the efficacy of this treatment after exposure to intravenously administered toxicants and have suggested positive effects to CVS toxicity. However, in the clinical setting, patients presenting to hospital with signs of cardiovascular compromise after poisoning have commonly ingested drugs orally. Clinical reports of ILE therapy are not as clearcut in their description of response to this treatment in case reports after oral poisoning. This project uses a rodent model to mirror the clinical oral poisoning scenario with various CVS toxicants and assesses the hemodynamic and pharmacokinetic responses to ILE therapy.

Andis Graudins MB BS, PhD, FACEM, FACMT.  
Professor of Emergency Medicine Research and Clinical Toxicologist,  
Monash University and Southern Health.  
Department of Emergency Medicine,  
Monash Medical Centre,  
Clayton Rd, Clayton, Vic, 3158  
AUSTRALIA  
Ph: +61395943193  
Fx: +61395946564  
[andis.graudins@monash.edu](mailto:andis.graudins@monash.edu)  
<http://www.med.monash.edu.au/medicine/mmc/research/sherg.html>

*Professor Nigel Bunnett, Monash Institute of Pharmaceutical Sciences, Parkville*

## **Bile Acids and Their Receptors: New Mechanisms of Steroid Signalling in the Nervous System**

Bile is episodically secreted from the gall bladder during digestion and is essential for the normal digestion and absorption of dietary lipids. Bile acids are then reabsorbed into the circulation and recycle back to the liver for secretion. Disruption of this normal entero-hepatic circulation of bile acids causes disease. Cholestatic diseases, where there is blockade in the normal secretion of bile, result in abnormally high circulating levels of bile acids, and are associated with intractable pruritus (itching) and analgesia. Reduced delivery of bile to the intestine causes constipation, whereas excess delivery causes diarrhoea. What is the mechanism of these abnormalities of sensation (itch/pain) and digestion (constipation/ diarrhoea)?

In addition to their role in digestion, bile acids are signalling molecules that can specifically regulate cells by activating nuclear and plasma membrane receptors. TGR5 is a newly-identified G protein-coupled receptor that has been implicated in major metabolic actions of bile acids, including control of energy balance and glucose homeostasis. We have discovered that TGR5 is expressed by primary spinal afferent and spinal neurons that transmit itch and pain, and by enteric neurons that control intestinal secretion and motility. We hypothesize that TGR5 mediates the well described effects of bile acids on sensory and digestive functions. Key unanswered questions include:

- Do bile acids control itch and pain by activating TGR5 on primary spinal afferent and spinal neurons?
- Do bile acids control secretion and motility in the intestine by activating TGR5 on enteric neurons or other cell types?
- Does TGR5 regulate the excitability of neurons by sensitizing ion channels?
- Is TGR5 a new target for digestive and sensory diseases?
- What is the mechanism by which diverse bile acids and structurally-related neurosteroids signal to neurons via cell-surface or nuclear receptors, and how is this signalling regulated?

These questions will be addressed by behavioural and physiological studies of mice lacking or overexpressing TGR5, including examination of primary neurons by electrophysiological approaches and signalling assays. Basic mechanisms of TGR5 signalling will be examined at the molecular and cellular level in model systems.

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## **Endosomes: A Legitimate Platform for the Signalling Train**

G protein-coupled receptors (GPCRs) comprise the largest family of cell-surface receptors that allow cells to detect and respond to an extraordinary diverse array of endogenous and environmental signals. GPCRs participate in all physiological processes and are essential mediators of disease. Most drugs target some aspect of GPCR signalling. In view of its fundamental importance, GPCR signalling at the plasma membrane has been extensively investigated and is under tight control. However, most activated GPCRs traffic from the cell surface to endosomes, a large and dynamic tubulo-vesicular network that ramifies throughout the cell. Although once regarded as a conduit for receptor trafficking back to the plasma membrane or to lysosomes, endosomes are now viewed as a key site of signal transduction. Emerging evidence suggests that the signals that originate from endocytosed receptors are distinct from those that arise from the plasma membrane, in terms of mechanism, duration and physiological outcome. Key unanswered questions include:

- What are the mechanisms of endosomal signalling?
- How is endosomal signalling switched on and off?
- What is the patho-physiological importance of endosomal signals?
- Do therapies that specifically target either endosomal or plasma membrane signalling offer more effective and selective treatments for diseases?

Projects are available that address these critical questions at the molecular and cellular levels and by using genetically-modified mice that permit examination of the patho-physiological importance of endosomal signals. Studies will investigate endosomal signalling and trafficking of receptors for neuropeptides that are major regulators of inflammation and pain signalling, including receptors for substance P, calcitonin gene-related peptide, and opioids.

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## **Proteases: Gnawing Away and Inflammation and Pain**

Proteases comprise >2% of the human genome and have remarkably diverse functions, ranging from the degradation of dietary proteins to the control of the coagulation cascade. Certain proteases that act at membrane surfaces can initiate or terminate signal transduction by cleaving ligands, receptors and ion channels. In particular, serine proteases and cysteine cathepsins that are activated during injury and inflammation can regulate cells by cleaving protease-activated receptors (PARs). Proteolytic activation of PARs on sensory neurons results in the release of neuropeptides that cause neurogenic inflammation and which transmit pain. Activated PARs also regulate the activity of ion channels, including members of the transient receptor potential (TRP) family of ion channels that are essential mediators of pain, resulting in hyperalgesia. Key unanswered questions include:

- What is the identity and cellular location of proteases that are activated in disease states?
- What is the causative role of proteases, PARs and TRP channels in chronic inflammation and pain?
- Does the detection of activated proteases offer new strategies for the early diagnosis of disease prior to irreversible organ damage, and are proteases predictive biomarkers of disease and therapeutic responsiveness?
- Do proteases that cleave PARs and ion channels at different sites activate distinct pathways of signal transduction?
- Given that proteolytic activation of receptors and channels is irreversible, what are the mechanisms that terminate these signals, and do abnormalities in these mechanisms cause disease?

Projects are available that address these questions at the molecular, cellular and whole animal level. The use of small molecule activity-based probes, coupled with proteomics, non-invasive whole animal imaging, and confocal cellular imaging, will permit the identification and localization of proteases that are activated in disease states. Studies of mice lacking proteases, PARs and channels will allow the determination of the importance of these mediators for chronic inflammation and pain. Basic molecular and cellular studies will examine PAR signalling and its regulation by specific proteases.

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**Supervisors: Dr. Lauren May and Prof. Arthur Christopoulos**

Drug Discovery Biology

Office: Room 325, Building 403

Contact: Ph: 99039067, e-mail: [arthur.christopoulos@monash.edu](mailto:arthur.christopoulos@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

Structural basis of allosteric modulator interactions at adenosine A<sub>1</sub> receptors.

Project Details:

G protein-coupled receptors (GPCRs) are involved in the majority of physiological and pathophysiological signal transduction mechanisms and, as such, represent the most commonly targeted group of proteins in terms of pharmaceutical therapeutics. The development of GPCR small molecule therapeutics typically involves targeting the endogenous ligand-binding (orthosteric) site, but it is now acknowledged that topographically distinct allosteric sites also exist on these receptors. Allosteric ligands represent an exciting approach to target GPCRs with enhanced spatiotemporal specificity and subtype selectivity<sup>1,2</sup>. Allosteric modulation of the adenosine A<sub>1</sub> GPCR (A<sub>1</sub>-AR) can promote cellular protection during conditions such as ischemia/reperfusion, epilepsy and chronic neuropathic pain<sup>3-6</sup>. However, the development of clinically relevant ligands is currently limited by a lack of structural knowledge regarding the location of the A<sub>1</sub>-AR allosteric site. *This project will use receptor mutagenesis to probe for the influence on the pharmacology of A<sub>1</sub>-AR agonists, antagonists and allosteric ligands.* The outcome of these studies will provide a greater understanding of the mode of allosteric ligand binding at the A<sub>1</sub>-AR and aid in the structure-based discovery of therapeutically relevant compounds.

Techniques that will be used in this project include:

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

Structural: Site-directed mutagenesis and molecular modelling, to develop a better picture of the adenosine receptor allosteric sites.

Molecular biological: Isolation and preparation of DNA, cell culture and cell transfection.

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**Supervisors: Dr. Lauren May and Dr. Celine Valant**  
Drug Discovery Biology  
Office: Room 325, Building 403  
Contact: Ph: 99039095, e-mail: [lauren.may@monash.edu](mailto:lauren.may@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

Understanding adenosine receptor signalling under hypoxic conditions

Project Details:

Conditions such as ischemia/reperfusion, epilepsy, inflammatory disorders and cancer are associated with transient or chronic intermittent hypoxia (TCIH). Within a hypoxic microenvironment, a critically important protective response involves a dramatic rise in the local concentration of endogenous adenosine. The actions of endogenous adenosine are mediated through the adenosine family of cell surface G protein-coupled receptors, A<sub>1</sub>-AR, A<sub>2A</sub>-AR, A<sub>2B</sub>-AR and A<sub>3</sub>-AR<sup>1-3</sup>. The A<sub>1</sub>-AR and A<sub>3</sub>-AR preferentially couple to G<sub>i/o</sub> proteins, decreasing cAMP production, whereas the A<sub>2A</sub>-AR and A<sub>2B</sub>-AR preferentially couple to G<sub>s</sub> proteins, increasing cAMP production. In addition to cAMP, adenosine receptors can also modulate intracellular calcium concentrations, stimulate ERK1/2 phosphorylation (pERK1/2) and, in excitable cells, promote potassium channel-mediated hyperpolarisation<sup>1</sup>. However, these signalling properties ascribed to adenosine receptors have been almost exclusively determined under normoxic conditions, despite the fact the receptors themselves are principally targeted for disorders associated with low oxygen! *We thus hypothesize that adenosine receptor signalling can be markedly altered within a hypoxic microenvironment, and knowledge of the mechanisms underlying such altered signalling will have a profound influence on treatment of diseases associated with TCIH.*

To test his hypothesis, the current project will compare the pharmacology of agonists at the A<sub>1</sub>-AR, A<sub>2A</sub>-AR, A<sub>2B</sub>-AR and A<sub>3</sub>-AR under normoxic and hypoxic conditions. The outcome of these studies will provide an understanding of the influence of hypoxia on adenosine-receptor mediated signalling within a hypoxic microenvironment.

Techniques that will be used in this project include:

Pharmacological techniques will include high throughput signalling assays for ERK1/2 phosphorylation, calcium mobilization and cAMP accumulation. You will also learn basic molecular biological procedures including cell culture and cell transfection. 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.

## References

- 1 Fredholm, B.B., et al., *International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and Classification of Adenosine Receptors--An Update*. Pharmacol Rev, 2011. **63**(1): p. 1-34
- 2 Hasko, G., et al., *Adenosine receptors: therapeutic aspects for inflammatory and immune diseases*. Nat Rev Drug Discov, 2008. **7**(9): p. 759-70.
- 3 Jacobson, K.A. and Z.G. Gao, *Adenosine receptors as therapeutic targets*. Nat Rev Drug Discov, 2006. **5**(3): p. 247-64.

**Supervisors: Dr. Celine Valant and Dr. Lauren May**  
Drug Discovery Biology  
Office: Room 325, Building 403  
Contact: Ph: 99039091, e-mail: [celine.valant@monash.edu](mailto:celine.valant@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

The effects of pH on adenosine A<sub>1</sub> receptor pharmacology

Project Details:

The nucleoside neurotransmitter, adenosine, plays a vital protective role in a variety of diseases that associated with transient or chronic intermittent hypoxia. These actions of adenosine are mediated through a family of cell surface G protein-coupled receptors, A<sub>1</sub>-AR, A<sub>2A</sub>-AR, A<sub>2B</sub>-AR and A<sub>3</sub>-AR. An important consequence of a hypoxic microenvironment is the occurrence of extracellular acidosis, yet the determination of drug pharmacology is routinely performed under conditions of physiological pH. The adenosine A<sub>1</sub>-AR, in particular, is an important therapeutic target for a variety of disorders linked to metabolic stress, and numerous agonists, antagonists and allosteric modulators have been described for this receptor. However, the impact of pH changes associated with hypoxic conditions on the pharmacology of these different classes of A<sub>1</sub> ligand are largely unknown. This project will thus investigate the influence of pH on the protonation status of histidine residues within the adenosine A<sub>1</sub> receptor and their interaction with adenosine receptor ligands. Histidine residues have been chosen because they are particularly sensitive to small, physiologically relevant, changes in pH. By determining the effects of pH at the wild type and at various A<sub>1</sub>-ARs where the histidine residues have been mutated, this project will provide new information that can be used to design improved ligands that function optimally under conditions of extracellular acidosis.

Techniques that will be used in this project include:

Pharmacological techniques will include high throughput signalling assays for ERK1/2 phosphorylation, calcium mobilization and cAMP accumulation. You will also learn basic molecular biological procedures including cell culture and cell transfection. 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.

## References

- 1 Fredholm, B.B., et al., *International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and Classification of Adenosine Receptors--An Update*. Pharmacol Rev, 2011. **63**(1): p. 1-34
- 2 Hasko, G., et al., *Adenosine receptors: therapeutic aspects for inflammatory and immune diseases*. Nat Rev Drug Discov, 2008. **7**(9): p. 759-70.
- 3 Jacobson, K.A. and Z.G. Gao, *Adenosine receptors as therapeutic targets*. Nat Rev Drug Discov, 2006. **5**(3): p. 247-64.

**Supervisors: Dr. Katie Leach and Prof. Arthur Christopoulos**

Drug Discovery Biology

Office: Room 337, Building 403

Contact: Ph: 99039067, e-mail: [arthur.christopoulos@monash.edu](mailto:arthur.christopoulos@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

Mutational Analysis of the Calcium Sensing Receptor

Project Details:

The human calcium sensing receptor (CaSR) is a Family C G protein coupled receptor (GPCR) that plays a pivotal role in extracellular calcium ( $\text{Ca}^{2+}_o$ ) homeostasis and parathyroid hormone (PTH) secretion. Over 200 clinically relevant polymorphisms have been identified in the CaSR, with many resulting in disorders characterised by an imbalance of PTH and/or extracellular  $\text{Ca}^{2+}$ .

Cinacalcet, a positive allosteric CaSR modulator prescribed for the treatment of primary and secondary hyperparathyroidism, was recently shown to successfully normalise serum  $\text{Ca}^{2+}$  levels in two individuals with loss-of-function CaSR mutations, suggesting that cinacalcet and other allosteric CaSR modulators could be invaluable in the treatment of disorders linked to CaSR mutations. Recent molecular modelling and mutagenesis studies have suggested that the binding site(s) for allosteric CaSR modulators is located predominantly in the transmembrane domains of the receptor, but no studies to date have identified which residues comprise the binding pocket relative to those residues that contribute to the transmission of the pharmacological (allosteric) effect; knowledge of these two properties is essential to enable the design of better CaSR-targeting drugs.

The current project will use a number of pharmacological assays to fully characterise the effects of alanine substitution of amino acids that have either previously been implicated in the binding of allosteric CaSR modulators, or that have been identified from a recent investigation into the effects of naturally occurring amino acids performed in our laboratory. This information will be used to aid the design of more accurate molecular models of the CaSR, which are currently lacking.

Techniques that will be used in this project include:

Structural: Site-directed mutagenesis and 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), intracellular calcium mobilization and inositol phosphate accumulation.

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.

**Supervisors: Dr. Rob Lane and Prof. Arthur Christopoulos**

Drug Discovery Biology

Office: Room 325, Building 403

Contact: Ph: 99039095, e-mail: [rob.lane@monash.edu](mailto:rob.lane@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

Molecular mechanisms of a novel allosteric modulator of the D<sub>2</sub>-like dopamine receptors

Project Details:

Since its discovery over 50 years ago, the neurotransmitter dopamine has been shown to play a vital role in a range of central nervous system functions including voluntary movement, reward, memory and learning. The actions of dopamine are mediated by its interaction with five G protein coupled receptors, the dopamine receptors D<sub>1-5</sub>R. Because dopamine is involved in such critical physiological processes, it is not surprising that many human disorders, including schizophrenia and Parkinson's disease, have been related to dopaminergic dysfunction.

To date, drug discovery at the dopamine receptors has focused on targeting the binding site for endogenous dopamine (the 'orthosteric' site). However, such an approach may not address issues of selectivity because this site is conserved across the entire dopamine receptor family. A new paradigm for GPCR drug design is the targeting of binding sites distinct from those of the endogenous ligand, called 'allosteric' sites. Recently, the first drug-like allosteric ligand of the dopamine receptor, SB269652, was described.

To determine the mechanism and site of action of this novel drug, we will mutate amino acids within the receptor that have been implicated in the binding of both orthosteric ligands or potential allosteric sites. These mutant receptors will be characterized using a number of state of the art functional assays. Information gained from this project and the recently published crystal structure of the dopamine D<sub>3</sub> receptor can be used to predict the mode of binding of this receptor and will be the starting point towards the development of novel allosteric drugs for this important therapeutic target.

Techniques that will be used in this project include:

Structural: Site-directed mutagenesis and molecular modelling, to determine the binding mode of SB269652.

Pharmacological: Receptor function will be quantified by downstream signalling using state-of-the-art, high throughput assays of extracellular signal regulated kinases (ERK1/2), intracellular cAMP, and resonance energy transfer techniques to detect interaction with  $\beta$  arrestin.

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.

**Supervisors: Dr. Rob Lane and Prof. Arthur Christopoulos**

Drug Discovery Biology

Office: Room 325, Building 403

Contact: Ph: 99039095, e-mail: [rob.lane@monash.edu](mailto:rob.lane@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

Stimulus bias at the dopamine D<sub>2</sub> receptor and its role in the treatment of schizophrenia

Project Details:

The dysregulation of dopaminergic signalling pathways has long been linked to schizophrenia, and almost all antipsychotic drugs block the dopamine D<sub>2</sub> receptor subtype as part of their mechanism of action. However, such approaches are hampered by serious side effects such as dystonia and Parkinson-like syndrome. An alternative approach is the use of ‘dopamine stabilisers’. These ligands are dopamine D<sub>2</sub> receptor partial agonists and were proposed to inhibit excess dopamine activity while retaining some low level dopamine tone thus reducing side effects. To date only one such agonist, aripiprazole (Abilify), is marketed as an antipsychotic drug. However, its mechanism of action is not clear since other dopamine partial agonists have failed to show clinical efficacy as antipsychotics.

We and others have proposed that a novel paradigm in drug design, termed “stimulus bias”, may explain the actions of drugs such as aripiprazole. This is a process by which different drugs acting at the same receptor promote multiple conformational states of the receptor linked to different functional outcomes. In the case of aripiprazole, whilst this drug is an *agonist* for the G protein mediated inhibition of cAMP production it is an *antagonist* for another signalling pathway involving the recruitment of  $\beta$  arrestin. It is this antagonism that has been linked to its antipsychotic action.

In this project we aim to determine the molecular mechanisms of action of aripiprazole. We will mutate amino acids within the receptor that have been implicated in the binding of drugs and the function of the receptor. The effect of these mutations on the binding and function of aripiprazole will be characterized using a number of state of the art functional assays looking at multiple signalling end-points. The information gained from this project can be used as a starting point for the design of novel, more effective antipsychotics.

Techniques that will be used in this project include:

Structural: Site-directed mutagenesis and molecular modelling, to determine the binding mode of aripiprazole.

Pharmacological: Receptor function will be quantified by downstream signalling using state-of-the-art, high throughput assays of extracellular signal regulated kinases (ERK1/2), intracellular cAMP, and resonance energy transfer techniques to detect interaction with  $\beta$  arrestin.

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.

**Supervisors: Dr. Meri Canals and Prof. Arthur Christopoulos**

Drug Discovery Biology

Office: Room 324, Building 403

Contact: Ph: 99039094, e-mail: [meri.canals@monash.edu](mailto:meri.canals@monash.edu)

Research Group:

Drug Discovery Biology

Theme:

Drug Discovery Biology

Project Title:

Ligand-biased signalling of the  $\delta$  opioid receptor

Project Details:

Opioid receptors (OR) belong to the superfamily of G protein-coupled receptors (GPCRs), which represents the largest receptor family in the human genome. ORs are widely expressed in the central nervous system and have been demonstrated to play key physiological roles in pain modulation and drug addiction. Interestingly, recent studies in knock-out mice suggest that the 3 subtypes of OR ( $\mu$ -,  $\delta$ -, and  $\kappa$ -) regulate different pain modalities. Despite the fact that opiates are the most effective analgesics available for the treatment of severe pain, their clinical use is restricted by unwanted side effects such as tolerance, physical dependence and respiratory depression. Generation of OR subtype-specific ligands has also not rendered novel therapeutic ligands with a better side effect profile than current opiates. A recent alternative approach to minimizing unwanted side effects is the possibility of exploiting the ability of certain ligands to stabilize specific receptor conformations to the expense of others and, as a consequence, elicit distinct signaling profiles. This property, termed “stimulus bias”, provides a potential means of directing the stimulus generated by activation of a GPCR towards a specific (beneficial) cellular response while avoiding deleterious responses associated with side-effects. This project will focus on identifying and quantifying stimulus bias at the delta-opioid receptor (DOR), which plays an important role in analgesia, gastrointestinal motility, mood and behavior as well as in cardiovascular regulation. To this aim, multiple opioid ligands will be characterized in a wide range of cellular signaling endpoints using complementary pharmacological and biochemical approaches. The systematic profiling of the signaling at the DOR will provide an essential tool in terms of identifying the mechanisms behind therapeutic responses versus unwanted side effects.

Techniques that will be used in this project include:

Pharmacological: Receptor signalling will be quantified by state-of-the-art, high throughput assays of cAMP accumulation, extracellular signal regulated kinases (ERK1/2), and  $\beta$ -arrestin recruitment. Receptor-G protein interaction will be monitored by determination of [ $^{35}$ S]GTP $\gamma$ S binding to activated G proteins.

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.

**Dr. Sebastian Furness, Dr. Denise Wootten, Dr. Bim Graham & Prof. Patrick Sexton**

Drug Discovery Biology

Office: Room 323, Building 404

Contact: Ph: 99039055, e-mail: [sebastian.furness@monash.edu](mailto:sebastian.furness@monash.edu)

**Research Group: Drug Discovery Biology**

**Theme: Ligand directed signalling bias and GPCR regulation**

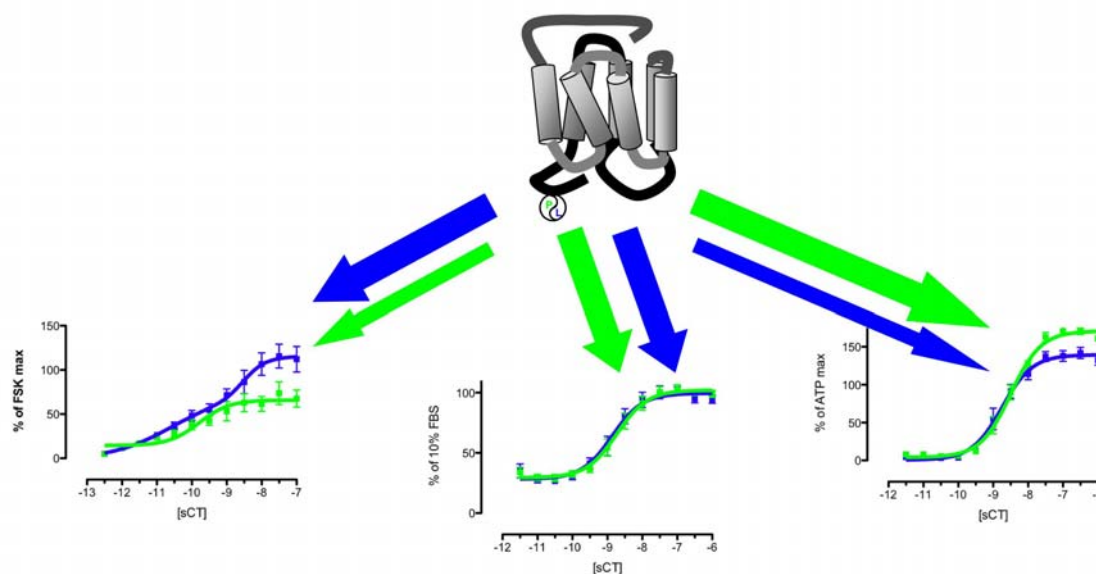
**Project Title:** Signalling bias of Calcitonin receptor polymorphs.

**Project Details:**

The Calcitonin receptor (CTR) is involved in calcium homeostasis by regulating its secretion in kidneys and the resorption of bone by osteoclasts in response to Calcitonin. The CTR is polymorphic in the C-terminal tail with a number of epidemiological studies correlating this polymorphism with osteoporotic risk. One previous study failed to uncover differences between CTR polymorphs (1). We have used several model cell backgrounds to analyse differences in signaling arising from these polymorphs. We have shown that there are a number of polymorphism-dependant changes in signaling efficacy toward adenylate cyclase, extracellular signal-regulated kinase (ERK) phosphorylation and intracellular calcium release (2). Further, we have demonstrated that these signaling biases are dependent on cellular background. This project will extend the existing signalling bias studies using bioluminescence resonance energy transfer (BRET) to examine the interaction between CTR and members of the arrestin and GRK families. In addition this project will utilize Europium criptate labelled CTR ligands to examine real time kinetics of ligand association with a view to multiplexing these measurements with real time second messenger activation.

(1) Wolfe et al. *Mutat Res.* 2003 522:93-105.

(2) Furness et al. Unpublished data.



**Dr. Sebastian Furness, Dr. Denise Wootten, Prof. Arthur Christopoulos & Prof. Patrick Sexton**

Drug Discovery Biology

Office: Room 323, Building 404

Contact: Ph: 99039055, e-mail: [sebastian.furness@monash.edu](mailto:sebastian.furness@monash.edu)

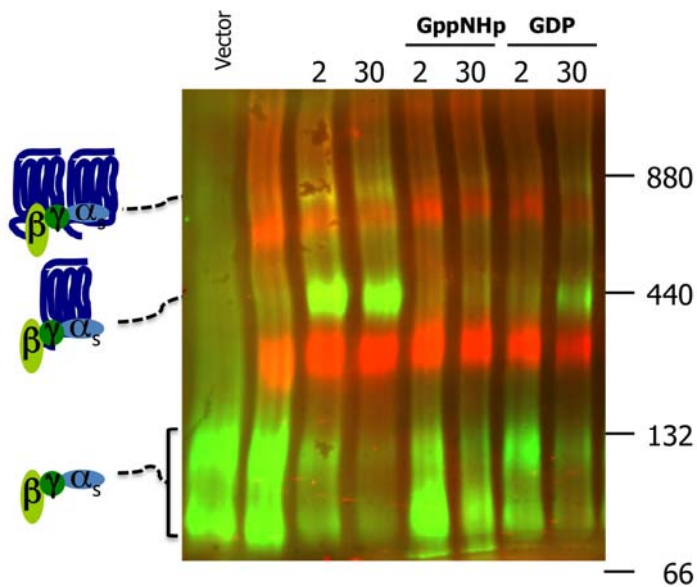
**Research Group: Drug Discovery Biology**

**Theme: The structural basis of GPCR binding and activation**

**Project Title:** Biochemical pharmacology of GPCR activation states.

**Project Details:**

We have recently applied high resolution native PAGE techniques to characterise the activation states of the Calcitonin receptor, a family B GPCR. These studies have allowed us to make new insights into the nature of receptor G-protein complex formation not previously examined and has allowed direct conclusions to be made about the stoichiometry of the components of this complex. This project will extend this type of biochemical pharmacology into other members of the GPCR superfamily including the GLP-1 receptor, which is implicated in diabetes therapeutics, the opioid receptors and muscarinic acetylcholine receptors. Each of these receptors displays unique signalling properties that will be investigated and has a series of unique tools that will allow us to probe novel aspects of their function.



**Dr. Denise Wootten & Prof. Patrick Sexton**

Drug Discovery Biology

Office: Room 344, Building 404

Contact: Ph: 99039088, e-mail: [denise.wootten@monash.edu](mailto:denise.wootten@monash.edu)

Research Group: Drug Discovery Biology

Theme: GPCR structure-function

**Project Title:** *Elucidation of allosteric binding sites and structural determinants of allosteric agonism and modulation at the glucagon-like 1 peptide receptor*

Project Details:

The glucagon-like peptide-1 (GLP1) receptor (GLP1R) is a family B GPCR that plays an essential role in nutrient mediated insulin release and as such is a therapeutic target for the treatment of type II diabetes (Drucker and Nauck, 2006). Unlike most GPCRs, the GLP1R is activated by four distinct endogenous GLP1 variants, as well as by further endogenous and exogenous ligands, which suggests the possibility of fine tuning the receptor by a spatio/temporal release of peptide signals. Recent developments in the GLP1R field include the discovery of non-peptide ligands, which bind at sites topographically distinct (allosteric) from the endogenous peptides. Allosteric ligands offer alternative routes into receptor activation that may be able to either modulate the existing peptide response and/or activate the receptor alone (May *et al.*, 2007). Whilst some insight has been gained in the interaction between the most documented of these compounds, compound 2, and the GLP1R peptide ligands (Koole *et al.*, 2010), a detailed understanding of the allosteric binding pockets and the transmission of cooperation between these and the orthosteric site is not known. This work will aim to elucidate the binding site(s) of small molecule ligands in the GLP1R and to provide a better understanding of how a modulatory signal is transmitted between allosteric and orthosteric binding pockets. Furthermore, for ligands, such as compound 2, that exhibit both selective modulation of endogenous peptide agonists and intrinsic agonism, molecular interactions which define these distinct features will be explored. The fundamental details of these events are required to be known in detail to advance allosteric drug design for this receptor target.

This project will include array of techniques to pharmacologically characterise a library of mutant and chimeric receptors, including cell culture techniques to generate and maintain stably transfected CHO FlpIn cell lines, signalling assays (cAMP and ERK, fluorescence based calcium signalling) and radioligand competition binding assays. The combination of experimental techniques, in addition to pharmacological and molecular modelling will give objective conclusions for the molecular basis of allosteric ligand binding at this receptor.

## OFF-CAMPUS PROJECTS: BAKER IDI (Pahran)

### ROLE FOR NITROXYL IN THE TREATMENT OF INFLAMMATION

Supervisors: Dr Karen Andrews<sup>1</sup>, Prof Jaye Chin-Dusting<sup>1</sup> & Dr Barbara Kemp-Harper<sup>2</sup>

<sup>1</sup>Baker IDI Heart & Diabetes Institute, Prahran

<sup>2</sup>Dept. Pharmacology, Monash University

Arteries play an important role in maintaining blood pressure as well as 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 atherosclerosis, 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 is an organic nitrate, which 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. A nitrate-free period is currently the only known protection and the underlying mechanisms of tolerance appear to be both multifactorial and not fully understood.

Nitroxyl (HNO), the reduced congener of NO $\cdot$ , may be a viable alternative to GTN since the HNO donor, Angeli's salt as it can dilate blood vessels and is resistant to the development of tolerance. Recent studies in our laboratory have also suggested that Angeli's salt can reduce adhesion of white blood cells (leukocytes) to the endothelium indicating HNO therapy may have additional benefits in treating patients with coronary artery disease.

This project aims to assess the mechanism underlying how HNO reduces adhesion using a novel vessel chamber technique which allows the imaging of leukocyte adhesion to the endothelium in real time. Additional techniques include real time-PCR, western blot analysis and oxidative stress assays.

**Dr Karen Andrews**

Vascular Pharmacology Group

Baker IDI Heart & Diabetes Institute

Phone: 8532 1294

[Karen.Andrews@bakeridi.edu.au](mailto:Karen.Andrews@bakeridi.edu.au)

**Dr Barbara Kemp-Harper**

Department of Pharmacology

Monash University

Phone: 9905 4674, Rm E147

[Barbara.Kemp@monash.edu](mailto:Barbara.Kemp@monash.edu)

## *Potential anti-inflammatory actions of AT<sub>2</sub> receptor ligands in vasculature*

**Prof Jaye Chin-Dusting, Dr Amanda Sampson, Dr Jennifer Irvine & Prof Robert Widdop**

Ang II is well known to stimulate both angiotensin AT<sub>1</sub> receptors (AT<sub>1</sub>R) and AT<sub>2</sub> receptors (AT<sub>2</sub>R). AT<sub>1</sub>R activation is responsible for the classical effects of Ang II such as vasoconstriction, but Ang II is also pro-inflammatory in the blood vessel wall. On the other hand, AT<sub>2</sub>R activation generally opposes AT<sub>1</sub>R effects (e.g. AT<sub>2</sub>R-mediated vasorelaxation or inhibition of AT<sub>1</sub>R-mediated signaling). However, the role of the AT<sub>2</sub>R has been much less studied in the context of vascular inflammation.

It is now recognized that local inflammation, evidenced by leukocyte recruitment and adhesion to the vessel wall, plays a critical role in early atherogenesis. In the current studies, we will test novel AT<sub>2</sub>R agonists, in a series of experiments that will delineate potential anti-inflammatory effects of AT<sub>2</sub>R in the vasculature.

Experiments will be performed using aortas, taken from young preatherosclerotic ApoE KO mice as well as AT<sub>2</sub>R knock out mice, which will be isolated and mounted in a vessel chamber. Vessels will be perfused with human whole blood and cell adhesion will be imaged in real time. In addition, pro-inflammatory mediators such as TNF-alpha will be used to prime the vessel prior to drug treatments.

The current project will be undertaken at the Baker IDI Institute (in Prahran), and will identify a potential vascular anti-inflammatory role of AT<sub>2</sub>R and explore underlying mechanisms. This project will involve animal work including:

- Surgical procedures
- In vitro vessel perfusion studies
- Video imaging vessel wall-cell interactions using a fluorescence microscopy
- Immunohistochemistry of tissue sections

**Prof Jaye Chin-Dusting**  
**Vascular Pharmacology Group**  
**Baker IDI Heart & Diabetes Institute**  
**Phone: 8532 1505**  
**jaye.chin-dusting@bakeridi.edu.au**

**Prof Robert Widdop**  
**Department of Pharmacology**  
**Monash University**  
**Phone: 9905 4858**  
**robert.widdop@monash.edu**

# Neuropharmacology Laboratory

**Professor Geoffrey Head**

Geoff.head@bakeridi.edu.au

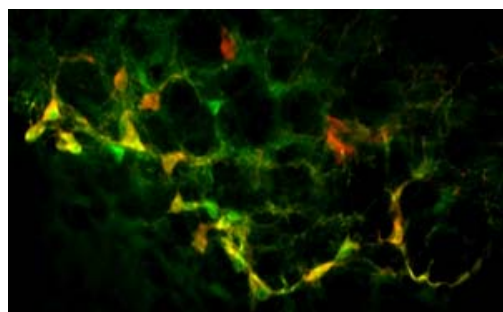
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**Research Focus:** The influence of the central nervous system on long-term blood pressure levels and the relationship between blood pressure and stress pathways in the brain is a major focus of the Neuropharmacology Lab's studies. Research in Neuropharmacology centres on cardiovascular neuroscience and fills a niche between the clinic and basic research. Work is carried out to understand the mechanisms that trigger cardiovascular diseases through environmental factors. Stress is a main area of investigation, and research is also being conducted on the effects on the central nervous system and control of the cardiovascular system of obesity and other metabolic disorders.

**Project 1: Role of neuropeptides in the amygdala mediating neurogenic hypertension**

**Supervisors: Professor Geoffrey Head and Dr Pamela Davern**

The Schlager hypertensive BPH/2J mouse has a neurogenic form of hypertension characterised by an exaggerated blood pressure response to stress and also a much greater cardiovascular circadian rhythm. Both of these are accompanied by a much greater activity of neurons in the amygdala and hypothalamus. The hypertension can be blocked by inhibiting the sympathetic nervous system (SNS) and also by specific lesions of the medial amygdala. Gene array studies have shown upregulated receptors for neuropeptide Y and S in the hypothalamus suggesting possibly low levels of peptide in these regions. In the current project we will investigate the contribution of these peptides in the hypertension in the Schlager mouse. The project will involve some animal surgery, experiments to measure blood pressure using radio telemetry, and during stress and also in the presence of agonists and antagonists for neuropeptide Y (BIIIE0246) and neuropeptide S (eg SHA-68). The animals will be perfusion fixed and processed for immunohistochemistry. The use of specific antibodies, fluorescence labelling



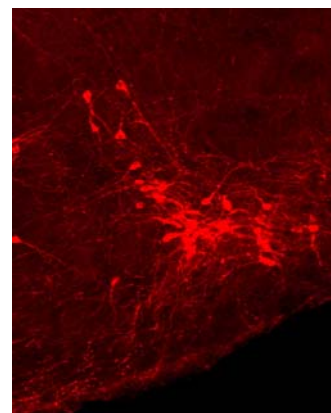
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and confocal imaging will then be used to identify the specific neuropeptides associated with stress and hypertension in these mice.

## **Project 2: Neurochemical subtype of stress responsive neurons leading to hypertension**

### **Supervisors: Professor Geoffrey Head and Dr Pamela Davern**

The hypertension in BPH/2J mice is likely due to over activity of the SNS driven by neurons in the medial amygdala that suggests that either the neurons are intrinsically more active or they receive more excitatory inputs. By co-localising stress activated neurons (c-Fos) with AT1 receptors and markers of NADPH activity (gp91phox, p47phox) or markers for CRF pathways, this study can describe the neurochemical subtype of neurons responsible for the 'over-responding' to stress. Alternatively, the greater levels of neuronal activation in the medial amygdala and hyperactive response to stress in BPH/2J mice might be associated with a loss of local inhibitory GABAergic neurons. Some neurogenic diseases describe a loss of GABAergic neurons in the amygdala that results in reduced inhibitory inputs leading to 'hyperexcitability' of the amygdala circuitry. We will also administer the anxiolytics to determine whether chronic inhibitory GABAA receptor activation will reduce hypertension and the sympathetic response to stress in BPH/2J mice. Neuropeptide Y has also been shown to induce anxiolytic effects and these fibres innervate GABA-containing neurons suggesting that the interaction may be important in the regulation of anxiety. This study will use immunohistochemical fluorescence to examine neurons either activated (c-Fos) or inhibitory (GABA) in consecutive sections and include labeling for AT1 receptors and neuropeptide Y. The project will also include some animal surgery to measure blood pressure with radio telemetry in conscious mice at rest and during stress.



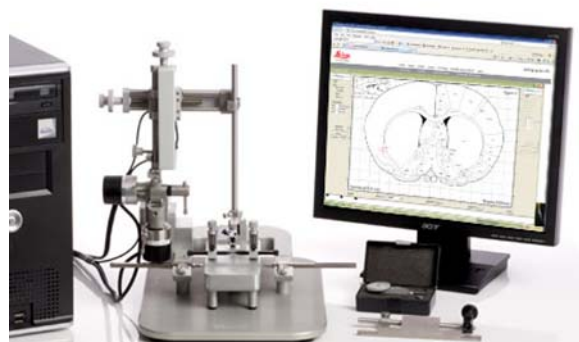
## **Project 3: Neurogenic hypertension in female Schlager hypertensive mice**

### **Supervisors: Professor Geoffrey Head and Dr Pamela Davern**

Studies in spontaneously hypertensive mouse show that hypertension in these mice is a neurogenic form, most likely driven by a specific part of the brain. Thus far our studies have been limited to male mice and the female of this strain is relatively under studied. However characterisation of the females could reveal further details of mechanisms driving the hypertension.

Therefore the current project will use radio-telemetry to examine the cardiovascular differences between the genders. We will perform a series of stress tests to assess if gender differences in cardiovascular response to stress. The metabolic profile of female mice will also be investigated using metabolic caging. Finally we will fix the brain for histology to establish any gender differences of brain regions driving the hypertension.

The project involves surgery, animal handling, computerised monitoring and analysis using special software and immunohistochemistry.



#### **Project 4: Role of brain pathways in chronic stress**

##### **Supervisors: Professor Geoffrey Head and Dr Pamela Davern**

There is now strong evidence to suggest that the sympathetic nervous system (SNS) makes a significant contribution to certain forms of hypertension. One of the suggested mechanisms through which the SNS may be “activated” in hypertension is through the renin-angiotensin system. We have recently demonstrated that a very low “subpressor” dose of Angiotensin given for several months to rabbits can produce a very modest increase in blood pressure but appears to amplify the effects of chronic stress. Using immuno-histochemistry, we have found that specific areas of the brain are activated by infusing angiotensin, an action which is mediated through areas of the brain that does not have a blood brain barrier. These central pathways appear to be “sensitised”, reflecting a type of positive feed forward CNS plasticity. We now wish to determine which areas of the brain and in particular the hypothalamus may be responsible for this effect on chronic stress. The project will involve some animal surgery, experiments to measure blood pressure before and during a relatively mild air jet stress and then perfusion fixation of the brain to process for immunohistochemistry. The use of specific antibodies, fluorescence and confocal imaging will then be used to identify brain regions and specific neurochemical involved in the response to acute and chronic stress.



## Heart Failure Pharmacology, Baker IDI Heart & Diabetes Institute

### **Nitroxyl, a relative of NO, is a naturally-occurring cardioprotective molecule**

Supervisors: A/Prof Rebecca Ritchie (Baker IDI) & Dr Barbara Kemp-Harper

Phone: 8532 1392

Email: [rebecca.ritchie@bakeridi.edu.au](mailto:rebecca.ritchie@bakeridi.edu.au)

The nitric oxide (NO•)/cGMP signalling system is as a powerful cardiac antihypertrophic mechanism. Nitroxyl (HNO), a novel redox sibling of NO•, has several therapeutic advantages for the treatment of cardiovascular diseases. We have shown that HNO prevents hypertrophy (abnormal pathological growth) and generation of superoxide in isolated cardiomyocytes. Excitingly, HNO also potentiates cardiac function, in contrast to NO•, via the cardiac calcium handling proteins, SERCA2a (sarcoplasmic reticulum Ca<sup>2+</sup>ATPase) and the ryanodine receptor RyR2. The activity and expression of these enzymes is abnormally affected in cardiac pathologies (LV hypertrophy, heart failure, diabetes), and together with the upregulation of ROS is recognised for as playing a causal role in the development of LV dysfunction. HNO thus is likely to be favourable for treating these cardiac pathologies.

We are now offering an exciting student research project in 2012, exploring whether HNO or related strategies represent novel pharmacotherapy for the prevention and treatment of myocardial dysfunction, induced by chronic LV hypertrophy, heart failure or diabetes. The project will examine whether the mechanisms by which HNO acutely enhances cardiac function in intact heart are different to those that prevent hypertrophy and elicit ROS suppression, and determine if acute or chronic HNO treatment is cardioprotective in isolated cardiomyocytes and the intact myocardium *in vivo* in settings of chronic cardiac impairment.

The scope of this project will be tailored depending on the student's abilities and interests. It will provide the opportunity for learning a range of techniques, including cell culture (cardiomyocytes and/or cardiac fibroblasts), physiological/pharmacological (e.g. isolated rodent hearts *ex vivo* or *in vivo* models of cardiac disease, for assessing cardiac function and blood pressure) biochemical (Westerns, ROS detection, ELISA, real-time PCR) and/or histological techniques. The outcome of this project will be definitive information regarding the mechanism(s) and effectiveness of HNO-mediated rescue of myocardial dysfunction. This project will be performed in **A/Prof Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran**. Ultimately, HNO-based strategies may offer new treatment options for cardiac disease, either alone or on top of standard care.

## **New strategies to rescue diabetes-induced cardiac dysfunction**

Supervisors: A/Prof Rebecca Ritchie (Baker IDI) & Dr Barbara Kemp-Harper

Phone: 8532 1392

Email: [rebecca.ritchie@bakeridi.edu.au](mailto:rebecca.ritchie@bakeridi.edu.au)

Diabetes is Australia's fastest growing chronic disease; one million Australians have been diagnosed, with close to one million more yet to be identified. Most of these patients will eventually die from cardiovascular causes. As diabetes induces left ventricular (LV) dysfunction, this increases the risk of death from heart failure in affected patients. Patients with diabetes are 2.4-fold more likely to develop heart failure, even when adjusted for age and coronary artery disease. Onset of heart failure occurs at a younger age in diabetic patients, with heart failure prevalence increased five- to eight-fold in middle-aged patients. New therapies for restoring cardiac function in the diabetic heart are thus highly desirable. In most forms of non-diabetic heart failure, systolic (contractile) dysfunction is the first and predominant functional abnormality. The aetiology of diabetic heart disease is distinct from other causes of LV dysfunction, as it is characterised initially by diastolic dysfunction, where relaxation of the cardiac muscle following contraction is prolonged. Diabetes-induced cardiac dysfunction is often exacerbated by underlying LV fibrosis (increased extracellular matrix deposition), hypertrophy (abnormal pathological growth) of cardiac myocytes, and excess generation of reactive oxygen species (ROS) such superoxide.

Our laboratory has demonstrated that antioxidant and/or ROS-suppressing approaches, as well as activation of cardioprotective signalling and negative regulators of LV hypertrophy, are beneficial for treating the cardiac complications of type 1 and type 2 diabetes in the intact heart. We are now offering an exciting student research project in 2012, exploring a novel potential therapeutic strategy for rescuing cardiac function and structure in the diabetic heart. This project will determine whether post-translational protein modifications induced by high glucose and implicated in insulin resistance play a causal role in the development of diabetic cardiomyopathy, and investigate whether pharmacological and/or gene-based strategies targeted at limiting these modifications can prevent diabetes-induced LV dysfunction and remodelling. The scope of this project will be tailored depending on the student's abilities and interests, and will provide the opportunity for learning a range of techniques, including physiological (e.g. isolated rodent hearts *ex vivo* or *in vivo* models of diabetic cardiac disease, for assessing cardiac function and blood pressure) biochemical (Westerns, ROS detection, ELISA), molecular (real-time PCR, Northern) and/or histological techniques. This project will be performed in **A/Prof Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran**. Ultimately, treatment strategies that may emerge from these studies may provide significant benefits alone or in combination with current standard care, to ultimately reduce progression to heart failure and death in diabetic patients.

## Targeting the anti-inflammatory protein Annexin-A1 for protection from myocardial infarction (heart attack)

Supervisors: A/Prof Rebecca Ritchie (Baker IDI) & Dr Barbara Kemp-Harper

Phone: 8532 1392

Email: [rebecca.ritchie@bakeridi.edu.au](mailto:rebecca.ritchie@bakeridi.edu.au)

Myocardial ischaemia, in which coronary blood flow is reduced, causes anginal chest pain, myocardial infarction (MI, also known as heart attack), and death. Myocardial infarction represents the major cause of death in Western societies, and in the next decade, this will expand to all corners of the world. The primary determinant of outcome from MI is the extent of cell death during and after ischaemia, from necrosis, apoptosis and/or autophagy. Restoration of blood flow (reperfusion) however is associated with the development of further cell death and impaired recovery of cardiac function, referred to as "reperfusion injury". Myocardial ischaemia-reperfusion induces an inflammatory response, with damage resulting from both infiltration of circulating inflammatory cells, as well as neutrophil-independent direct actions on myocardium and endothelium (including  $Ca^{2+}$  overload, ROS generation and impaired mitochondrial regulation all contributing mechanisms to cell death. In addition, there is incomplete recovery of LV function. Together these phenomena contribute to increased risk of ischaemic cardiomyopathy, heart failure and death. Novel treatment strategies that protect against multiple mechanisms of MI injury will have major clinical impact.

The therapeutic potential of the anti-inflammatory mediator annexin-A1 (ANX-A1) has been recognized in a range of systemic inflammatory disorders. Importantly, we have shown that ANX-A1 has powerful protective actions against cardiac injury and loss of LV contractile function. We are now offering an exciting student research project in 2012, exploring the potential for mimetics of ANX-A1 to represent potential new pharmacotherapy for treating cardiac inflammatory disorders such as ischaemia-reperfusion (I-R) injury. The project will test the hypothesis that ANX-A1 represents a novel modulator of myocardial viability and LV contractile function following ischaemia-reperfusion, and will seek to investigate the cardioprotective function of endogenous ANX-A1 in I-R injury, the receptors responsible for cardioprotection elicited by ANX-A1 and its mimetics, and examine the potential therapeutic opportunities offered by exogenous ANX-A1 mimetics after I-R injury in the intact heart.

The scope of this project will be tailored depending on the student's abilities and interests. It will provide the opportunity for learning a range of techniques, including cell culture (cardiomyocytes), physiological/pharmacological (e.g. isolated rodent hearts *ex vivo* or *in vivo* models of ischaemic cardiac disease, for studying impact on cardiac function and structure) biochemical (Westerns, ROS detection, ELISA, real-time PCR) and/or histological techniques. These studies will provide insight into ANX-A1-mediated rescue of myocardial viability and function after I-R injury in the intact heart, and the mechanisms involved. This project will be performed in **A/Prof Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran**. Development of therapeutic strategies for treating myocardial infarction after an unplanned ischaemic event (while reperfusion injury is still evolving), alone or concurrent with standard care, will ultimately reduce progression to heart failure and death in affected patients.