

# Cardiovascular Research at Monash Physiology (Part 1)



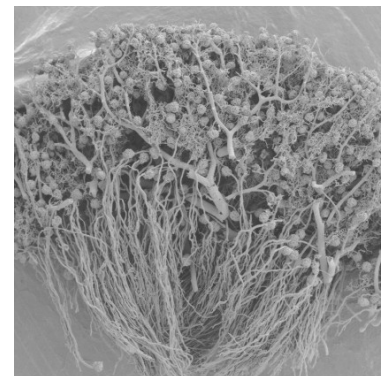
Cardiovascular Research at Monash University is represented by a group of over 50 research staff and students and is lead by Professor Warwick Anderson; Head of the Department of Physiology, and of Monash's School of Biomedical Sciences (see photo left). This month part I features the integrative renal and cardiovascular laboratories. Next month part II features other aspects of vascular disease, including diabetes, heart disease, and obesity.

[Cardiovascular research at Monash Physiology](#) focuses on the causes of the primary cardiovascular disease processes of hypertension and vessel wall dysfunction and damage. Given the prevalence of cardiovascular disease and hypertension world-wide, it is remarkable that more than 100 years since the introduction of the sphygmomanometer, we still have only a rudimentary understanding of

how the body regulates arterial blood pressure. It is essential to understand this if we are to understand what causes high blood pressure and thus how it can be prevented or targeted more effectively. For vessel dysfunction, much more progress has been made in recent years, mainly through advances in our understanding of endothelial cell and vascular smooth muscle biology; but the voyage of discovery has only just begun. What seems clear is that the integrative biosciences and the approaches known as systems biology will play increasingly important roles.

## Hypertension

Our primary focus is on the kidney in hypertension, including the study of neural regulation of renal function and neural plasticity, on genetic and environmental factors affecting renal development, on the roles of local paracrine factors, and on the filtration/vessel interface. We take as our underlying hypothesis that long term blood pressure levels depend in a major way on the kidney, with its ability to quickly raise or lower pressure through the powerful pressure-natriuresis mechanism. Whatever else changes as hypertension develops, there must be some alteration to this relationship for the pressure to remain elevated while blood fluid balance is maintained. This question can really only be addressed fully in vivo. Some of our specific projects are described below.



*Figure: Scanning electromicrograph of a cast of the renal vasculature. (scale 1mm). Denton et al CEPP, 2004*

## Renal medullary mechanisms

Roger Evans, Gabriela Eppel, Michelle Kett, Kate Denton

The renal medullary circulation has emerged as a major factor in long-term control of arterial pressure. Our work has focussed on understanding how hormonal and neural factors interact in the control of medullary perfusion.

Our major recent findings include:

- Medullary blood flow is relatively insensitive to a range of vasoconstrictor factors, including the renal sympathetic nerves, angiotensin II and endothelin-1. Both structural (vascular architecture) and functional (counter-regulatory paracrine factors) factors underlie this.
- Medullary blood flow is more sensitive than cortical blood flow to the vasoconstrictor effects of vasopressin. This appears to be due to vasopressin-induced release of epoxyeicosatrienoic acids in the cortical circulation.
- There are complex interactions between neural and hormonal (eg angiotensin II and vasopressin) mechanisms in control of medullary blood flow.



*Students and Collaborators: Dr Niwanthi Rajapakse (Medical College of Wisconsin), Dr Erika Boesen (Medical College of Georgia), Dr Lisa Duke (Melbourne law firm), Assoc Prof Rob Widdop (Pharmacology, Monash), Dr John Haynes (Pharmacy & Pharmaceutical Biology, Monash), Prof Kerry Hourigan and Dr Greg Sheard (Mechanical Engineering, Monash University); Prof Arthur Lowery and Dr Malin Premaratne (Electrical Engineering, Monash), Assoc Prof Geoff Head and Sandra Burke (Baker Heart Institute,), Assoc Prof Simon Malpas (Auckland).*

*Pictured: Cardiovascular Research Group at Monash representatives*

*Left Back: Chai Ling Leong, Amelie Dinsdale, Gavin Dyson, Sarah Richerson, Jessica Cox, Dr Roger Evans, Assoc Prof Helena Parkington, Hommira Bashari.*

*Left front: Dr Sharyn Fitzgerald, Leah-Anne Ruta, Dr Michelle Kett, Dr Amany Shweta, Dr Gabriela Eppel.*

## Renal regulatory mechanisms in glomerular pressure and tubulo-glomerular feedback control

Kate Denton and Warwick Anderson

A key factor in long-term blood pressure regulation via the pressure natriuresis mechanism is control of glomerular filtration pressure within precise limits, achieved by the physiological regulation of pre- and post-glomerular vascular resistance. However, this is a difficult area to study, since it can be studied only in vivo, and by micropuncture measurements of the glomerular capillary pressures. Kate Denton's lab is one of only a handful in the world that has mastered the technically demanding methodologies and, as well, she has maintained a colony of rabbits with glomerular accessible on the surface of the kidney (the only such colony world-wide). Her work has made major contributions to our understanding of differential afferent/efferent regulatory mechanisms, to the role of angiotensin II and renal nerves, and to tubulo-glomerular feedback regulation of GFR.

*Students and Collaborators: Amanda Sampson, Professor Jurgen Schnermann (NIH, USA), Dr Russell Brown (Uppsala University, Sweden).*

## Renal nerves

Kate Denton, Amany Shweta, Helena Parkington, Harry Coleman, Gaby Eppel, Warwick Anderson

Kate, Helena, Warwick and their teams, with Susan Luff, have made several major new findings that redefine our understanding on the role of nerves in the kidney. In brief, these findings are:

Two different types of sympathetic nerves innervate renal effectors

One type runs almost exclusively to the pre-glomerular resistance vessels, the other (Type II) is distributed to both pre-and post-glomerular vessels. The neurotransmitter NPY is only present in Type II renal nerves.

This allows differential regulation of glomerular filtration pressure. Kate has shown that, for example, mild hypoxia activates only the latter nerves, and this helps maintain GFR even during quite strong renal vasoconstriction. Severe hypoxia, however, activates also the nerves that supply only pre-glomerular vessels, causes a profound vasoconstriction and falling GFR.

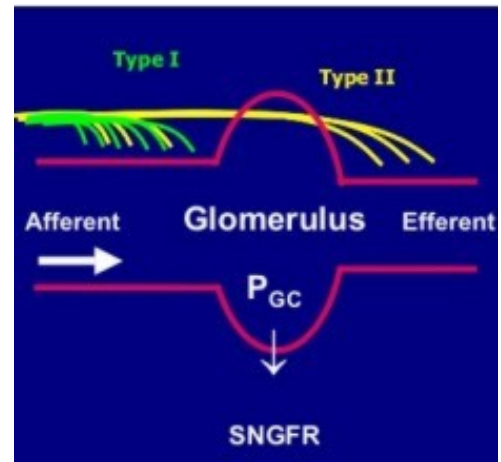
The innervation of the renal vasculature is plastic – changes in the renin-angiotensin system affect the density of innervation of the kidney. This has important functional consequences. We have shown that renal nerve stimulation causes a markedly more pronounced vasoconstriction of renal resistance vessels in rats with prolonged angiotensin II infusion.

NPY contributes to vascular responses to electrical stimulation of the renal nerves in both the cortex and medulla, not only directly via Y1-receptor mediated vasoconstriction, but more profoundly by modulating  $\alpha$ 1-adrenoceptor mediated vasoconstriction.

A novel pro-constrictor ion channel has been identified in arterioles. The spasm-like constriction evoked by neurotransmitters may involve this channel, and be altered in disease. We are in the process of characterizing the properties of this channel.

*Students and Collaborators: Amelie Dinsdale, Dr Susan Luff (Monash Micro-imaging), Assoc Prof Geoff Head (Baker Heart Institute).*

*Fig. 4 Schema of the distribution of the 2 populations of nerves innervating the glomerular arterioles (Denton et al, CEPP 2004).*



## Renal oxygenation

Rogers Evans, Paul O'Connor, Warwick Anderson

We recently demonstrated that reduced blood flow in the renal cortex could decrease tissue PO<sub>2</sub> in the renal medulla, even when medullary perfusion is maintained. We believe this finding has important implications for the prevention and management of acute renal failure, which is primarily a condition of hypoxic damage in the outer medulla. It seems likely that the dependence of medullary PO<sub>2</sub> on cortical perfusion results from the shunting of oxygen from arteries to veins in the kidney. This, in part, led Paul O'Connor to develop the novel hypothesis that preglomerular arterial-venous oxygen shunting is a structural antioxidant defence mechanism in the kidney.

*Students and Collaborators: Dr Paul O'Connor (Medical College of Wisconsin), Dr Grant Drummond (Department of Pharmacology, Monash University).*

### *Recent Papers from Cardiovascular research at Monash Physiology*

1. Denton KM, Shweta A, Flower RL, and Anderson WP. Predominant postglomerular vascular resistance response to reflex renal sympathetic nerve activation during ANG II clamp in rabbits. *Am J Physiol Regul Integr Comp Physiol* 287: R780-786, 2004.
2. Evans RG, Eppel GA, Anderson WP, and Denton KM. Mechanisms underlying the differential control of blood flow in the renal medulla and cortex. *J Hypertens* 22: 1439-1451, 2004.
3. O'Connor P M, Kett MM, Anderson WP, and Evans RG. Renal Medullary Tissue Oxygenation is Dependant on both Cortical and Medullary Blood Flow. *Am J Physiol Renal Physiol*, 2005. In Press
4. Parkington HC, Dodd J, Luff SE, Worthy K, Coleman HA, Tare M, Anderson WP, and Edgley AJ. Selective increase in renal arcuate innervation density and neurogenic constriction in chronic angiotensin II-infused rats. *Hypertension* 43: 643-648, 2004.

# Cardiovascular Research at Monash Physiology (Part 2)



This month we continue with research at Monash Physiology focusing on the role of the kidney in hypertension, fetal programming leading to hypertension, nephron endowment, diabetes and the heart.

## **Fetal programming of adult hypertension**

**Kate Denton, Marelyn Wintour, Marianne Tare, Helena Parkington, Michelle Kett**

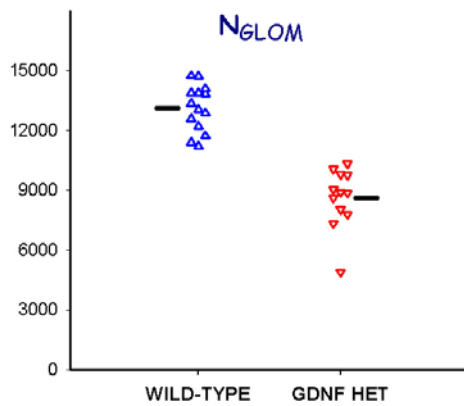
Marelyn's and Kate's teams are investigating how fetal environmental events may "program" the blood pressure regulatory systems so that hypertension develops in adulthood. Marelyn published pivotal studies on the effects of fetal exposure to steroids on development of hypertension, and has extended these studies with Karen Moritz, Miodrag Dodic and Rob DeMatteo following her move to Physiology in 2002. Currently Marelyn is an Honorary Professor, in the Department of Physiology. Kate Denton with Rebecca Flower (research assistant), and Dev Maduwegedera (PhD student) has developed an exciting new model, studying the blood pressure of offspring of rabbit mothers with renal wrap hypertension. These studies show that chronic kidney disease associated with high blood pressure adversely effects maternal extracellular fluid homeostasis during pregnancy and exposes the fetus to an unfavourable environment in which to develop. Recent results suggest that fetal development of the renal sympathetic nerves has been altered and that the functional response is increased in the offspring prior to the onset of hypertension.

Identifying other potential hazards, Marianne Tare, Helena Parkington and group has shown that intrauterine growth restriction in sheep markedly influences vascular reactivity and mechanical wall properties in the coronary vasculature in the offspring. They have also shown that vitamin D deficiency in utero in rats results in hypertension in the offspring, accompanied by significant vascular endothelial dysfunction.

*Students and collaborators: Kristen Bubb, Dewaki Maduwegedera, Adelle McArdle, Dr Karen Moritz and Dr Jane Black (Anatomy & Cell Biology, Monash University), Dr Ruth Morley and Sonya Grover (Murdoch and Children's Medical Research Institute), Dr Clare Roberts (Research Centre for Reproductive Health Department of Obstetrics and Gynaecology, University of Adelaide).*

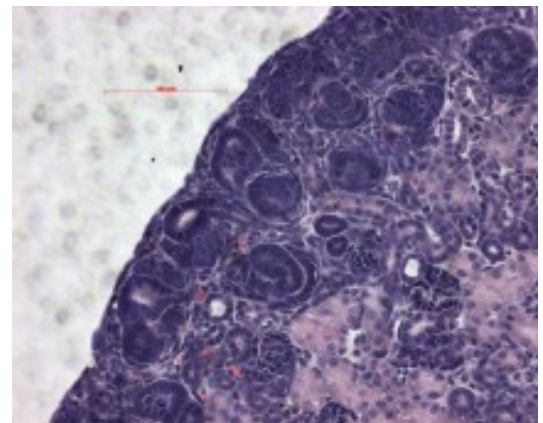
## Nephron endowment

Michelle Kett, Amany Shweta, Roger Evans, Warwick Anderson



There is increasing evidence that the number of nephrons that an individual possesses, so-called nephron endowment, may be a major factor in setting arterial blood pressure levels. In fact, several of the environmental factors described above are thought to have a direct effect on the expression of genes involved in nephrogenesis in the foetus. Michelle Kett (Foundation for High Blood Pressure Research Fellow) has described that mice heterozygous for the GDNF gene, and who are born with a 30% fewer nephrons, develop hypertension in old age (Figure 1). This is similar to studies to studies from humans showing reduced nephron numbers in hypertensive patients (see Keller et al New Eng J Med 2003).

With PhD Student Leah-Anne Ruta, she is now studying whether this reduction in nephron capacity renders the mice more prone to the hypertensive effects of high salt, and other environmental circumstances. Using a mouse model of neonatal unilateral nephrectomy Michelle is also examining the mechanisms whereby the timing of nephron loss leads to an increased risk of cardiovascular disease. Fig 2 shows the relative immaturity of the mouse kidney at birth providing an excellent model to examine the role of reduced nephron number at an early age.



*Pictured left: Figure 1 Glomeruli number from GDNF mice*

*Pictured right: Figure 2 Renal cortex from 1 day old Mouse*

Other studies, are investigating the molecular basis of the developmental programming of reduced nephron endowment in collaboration with the Department of Anatomy & Cell Biology, Monash.

*Students and collaborators: Leah-Anne Ruta, Chantal Hoppe, Dr Luise Cullen-McEwen, Dr Sharon Ricardo (Monash Immunology and Stem Cell Laboratories), Dr Karen Moritz and Professor John Bertram (Department of Anatomy and Cell Biology, Monash University), Professor Melissa Little (University of Queensland).*

## Diabetes

**Helena Parkington, Marianne Tare, Harry Coleman, Sharyn Fitzgerald**

This group uses detailed electrophysiological techniques, coupled with molecular biology and haemodynamic measurements in conscious and anaesthetized animals to probe the details of the disturbances that complicate diabetic arteries. We have found that

- The effects of diabetes are not uniform throughout the vasculature, especially in relation to the nature of the endothelial factor impaired.
- Diabetes interferes with endothelial potassium channels that play a key role in normal vascular endothelial vasodilator function.
- In vivo and subsequent in vitro studies suggest the involvement of reactive oxygen species whose influence, again, appears to differ in different vascular beds.
- The stiffening of arteries seen in diabetes can be rescued by new agents that prevent or reverse the destructive cross-linking induced by excess circulating glucose.
- The role of fat cells in the vascular complications of diabetes is also under study.
- Investigating how hyperglycaemia is affecting vessel function, concentrating on the role of endothelial factors by utilising genetic models in mice, particularly NOS knockouts.

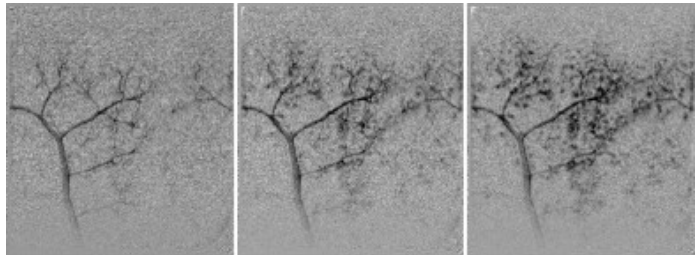
Additionally, Dr Amanda Edgley, an NHMRC Industry Fellow, currently working at Astra Zeneca, Sweden, will return to Melbourne in the New Year. Amanda has been utilising mouse models of diabetes and obesity to study vascular and cardiac function.

*Students and Collaborators: Jyosthna Rama, Dr Barbara Kemp (Department of Pharmacology, Monash).*

## The heart

**Igor Wendt, James Pearson**

Assoc. Prof. Igor Wendt (Deputy Head, Department of Physiology) is investigating sex differences in cardiac physiology, particularly the effects of sex hormones on myocardial calcium movements and on the development of cardiac hypertrophy. Findings to date indicate that oestrogen reduces calcium levels in the female heart, and is also able to reduce the responsiveness of heart cells to stimuli that induce abnormal cellular growth (hypertrophy). This supports the idea that oestrogen is able to act at the myocardial cellular level to exert potential cardio-protective actions.



James Pearson is a Monash Synchrotron Senior Research Fellow and leads one of only two research groups in the world that uses synchrotron x-ray radiation to investigate molecular mechanisms of myocardial contraction in vivo, to pursue the basic mechanisms that malfunction in ischemia and heart failure. He currently uses the SPring-8 synchrotron in Japan, but will work with the Australian synchrotron located adjacent to the Monash Clayton campus after it opens next year. This unique research brings together traditional cardiac function measurements of whole heart contractility and x-ray diffraction recordings of cardiac myosin-actin interactions in localised regions of the heart wall. This approach enables micro-level investigations of regional dysfunction. This work is currently being extended to included study of real-time renal vascular reactivity (see figure 3).

Fig 3 Real-time contrast microangiography of rat kidney in vivo using Synchrotron technology. The 3 images show different time-points after administration of contrast bolus into the renal circulation. Image courtesy of Gabriela Eppel and James Pearson.

*Students and Collaborators: Ruchi Patel, Dr Rebecca Ritchie (Baker Institute), Associate Professor Lea Delbridge and Dr Claire Curl (Physiology, Melbourne University). Prof. Mikiyasu Shirai (Hiroshima Intl. University) and Dr Kenji Kangawa and Dr Hiroyuki Suga (National Cardiovascular Center, Japan).*