Decoding complex signals

In this discussion, Dr Donald Maurice describes collaborative research into the underlying causes of vascular disease and identification of specific molecular interactions that govern dysfunctional cell behaviour.

Could you outline the objectives of your work on the role of cyclic nucleotide (cAMP and cGMP) compartmentation and cyclic nucleotide signalling in human vascular cells?

A cursory reading of any medical textbook presents us with a dilemma: although cAMP and cGMP are small, water soluble, and should diffuse rapidly throughout the cell, numerous hormones and drugs use these to regulate myriad cellular processes with exquisite specificity. Recent work, including ours, has shown that selective decoding of the information provided by individual hormones or drugs requires formation of several distinct signalling complexes, in which the proteins required for a given cellular function are tethered together via protein-protein interactions (signalosomes); and specific subcellular targeting of these complexes in cells (compartmentation).

Our work confirms that cAMP and cGMP signalling compartments form in cardiovascular cells and supports the concept that the spatial and temporal actions of cAMP and cGMP are dependent largely on their actions within the compartments. As a corollary, our research supports the therapeutically-relevant proposition that controlling events selectively within individual compartments provides increased specificity over traditional approaches, which have aimed to influence cellular cyclic nucleotide signalling globally.

What drew you to focus your studies on the enzymes that inactivate cAMP and cGMP, the cyclic nucleotide phosphodiesterases (PDEs)?

By hydrolysing and thus inactivating cyclic nucleotides, PDEs regulate the amplitude and duration of cyclic nucleotide signals, and hence their effects in cells. The superfamily of PDE enzymes contains 11 families, PDE1 to PDE11, and counts more than 60 separate enzymes. The pharmaceutical industry has long recognised that PDEs represent very good drug targets and since the 1980s several such drugs have entered the market.

Why did you to turn your attention to enzymes of particular PDE families?

Vascular endothelial and smooth muscle cells cooperatively control the functions of blood vessels in both health and disease. Indeed, they act adaptively to control blood pressure, to ensure appropriate blood supply and each participates in the repair of damaged tissues and organs. These cells can also act mal-adaptively to promote atherosclerotic plaque rupture or restenosis which both increase cardiovascular morbidity and mortality. Using selective pharmacological inhibitors of PDE1, PDE3, PDE4 or PDE5 family enzymes, we have shown that these enzymes may represent valid therapeutic targets through which to increase the adaptive functions of arterial endothelial or smooth muscle cells, or to reduce their mal-adaptive effects.

You have shown that cyclic nucleotide signalosomes containing the cAMP or cGMP-activating proteins also contain PDEs. What is the significance of this?

There are a limited number of cAMP or cGMP-activating proteins in cells – the protein kinases (PKA, PKG), which phosphorylate substrate proteins, and the EPACs (EPAC1 and EPAC2), which increase levels of activated Rap1, a small cytosolic protein that acts like a cellular switch. Since virtually all cyclic nucleotide signalosomes made by either arterial endothelial or smooth muscle cells contained these same proteins, it was unclear how individual signalosomes could selectively alter individual cell functions. Recently, we showed that the various cAMP-signalosomes each contained unique PDEs. Indeed, we showed that this integration of unique PDEs into the signalosomes was responsible for compartment-specific signalling. Most interestingly, we demonstrated that, by removing a PDE from a given signalosome, we could abrogate the ability of the signalosome to control local activation of the cAMP-activated proteins and their effects on function.

Finally, why is your approach to the study of human vascular endothelial cells innovative?

Our studies, which were only possible due to collaborations between our laboratory and those headed by Drs Miles Houslay and George Baillie in Glasgow, and Dr Manuela Zaccolo in Oxford, have allowed us to make several potentially important observations. First, they showed that it might be possible to alter a selected cellular function by targeting solely the PDE protein/activity present within its signalosomes – which can represent as little as 10 per cent of total PDE activity – rather than all of its cellular activity. Second, they confirmed and extended a hypothesis proposed by Professor Laurence Brunton that two substances can increase cyclic nucleotide levels equally and yet produce markedly different effects. Third, since we showed that signalling proteins not normally considered when thinking of cyclic nucleotide signalling (for example, those involved in lipid signalling) were also present in cyclic nucleotide signalosomes, our work identifies these signalosomes as potential points of signal convergence and integration in cells.
LOCATION, LOCATION, LOCATION

Research into the specific functions of signalling molecules and their organisation in cardiovascular tissues at Queen’s University aims to identify targets for safer treatments of conditions such as atherosclerosis.

ATHEROSCLEROSIS, THE HARDENING of the arteries caused by the build up of lipid plaques on the artery walls, restricts blood flow to the heart and is the leading cause of heart attacks, vascular disease and stroke in industrialised countries. It is often treated via angioplasty, a non-surgical procedure that reopensthe blocked blood vessel and is usually accompanied by stenting, where a wire mesh tube is inserted to keep the blood vessel open.

In many cases, restenosis – the re-narrowing of the arteries or large blood vessels – occurs some months after an apparently successful angioplasty and stenting procedure. It is thought to be caused by blood clotting at the site of treatment or a proliferation of the smooth muscle cells that line the walls of blood vessels, due to an overzealous healing response to trauma by the body.

Dr Donald Maurice, Director of the Cardiac, Circulatory and Respiratory (CCR) Research Programme and Professor of Biomedical and Molecular Sciences at Queen’s University in Kingston, Ontario, investigates the roles of second messenger molecules in angiogenesis, atherosclerosis and restenosis through their interactions with human vascular cells, including arterial endothelial cells and arterial smooth muscle cells. His laboratory is currently exploring the hypothesis that the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are implicated in pro-atherogenic responses by cells; and that by specifically targeting their signalling mechanisms in vascular cells it may be possible to treat atherosclerosis and restenosis more effectively.

THE MAURICE LABORATORY

Second messengers are molecules that relay amplified signals from receptors on the cell surface to target molecules inside the cell. They relay the effects of growth factors and hormones, such as adrenaline, that otherwise cannot pass through the outer cell membrane, and cause a physiological change that alters cellular activity. The cyclic nucleotides, cAMP and cGMP, influence the functions of most cells in the body; their action is, however, selective and varies according to the type and location of the cell. In the cardiovascular system, cAMP and cGMP regulate many functions, including cell adhesion, migration and proliferation, all of which underlie cardiovascular diseases. The Maurice laboratory is therefore investigating how cAMP and cGMP achieve selective regulation of the cardiovascular system, and how dysfunctional regulation plays a part in cardiovascular disease.

Maurice focuses on the interactions that allow co-localisation of the numerous proteins that must come together and collaborate for cAMP or cGMP signalling in cardiovascular cells to be regulated efficiently. These signalling protein complexes are called signalosomes and they function in discrete compartments in cardiovascular cells to give rise to the specificity with which cAMP and cGMP regulation works: “Put simply, specificity arises when cells form several distinct cyclic nucleotide signalosomes in which the proteins required to regulate a given cellular process are allowed to co-localise, interact, and act with common purpose – it’s all about location, location, location!” he reveals.

The actions of a superfamily of enzymes, the cyclic nucleotide phosphodiesterases (PDEs), which can disrupt cAMP and cGMP signalling spatially and temporally, have long interested Maurice, and he is convinced that not only do they play an important role in governing signalling, but that they also have potential as targets for therapeutics: “When I first started working on PDEs as a graduate student with the late Dr Richard Haslam at McMaster University, I was keen to show that PDEs were more than involved in cleaning up after the cAMP or cGMP messengers were done with their important activities, but rather that they were integral partners in regulating these activities. With the assistance of my collaborators and other colleagues I think we’re well on our way to doing just that.”

NURTURING YOUNG TALENT

For Maurice, the great benefit of having his own research laboratory and also his position as Director of the CCR Research Programme at Queen’s University is that they afford opportunities to nurture younger researchers: “The best part of my ‘job’ is the ability to mentor graduate students and postdoctoral fellows,” he muses. “Much of my time is spent interacting directly with trainees, either in the laboratory when conducting experiments, in the office when writing manuscripts, or at...
PDE enzymes are known to present good targets for drug development, especially those of the PDE5 family.

MULTIDISCIPLINARY APPROACHES

The Maurice laboratory employs multidisciplinary approaches to focus on the molecular bases of the multiple cyclic nucleotide-mediated effects in vascular tissues. Indeed, the group has used biochemical, pharmacological and cell biological methods to determine how cAMP or cGMP signalosomes integrate numerous proteins and regulate their functions, and to establish how the structures and regulation of human arterial endothelial cells become altered to effect angiogenesis, or so that atherosclerotic lesions develop.

Another important tool in the group’s repertoire is the use of peptide arrays to identify peptides that can be used to interfere with the integration of selected PDEs into individual signalosomes. These ‘disruptor peptides’ allow targeting of events within individual signalosomes as opposed to targeting PDE catalytic activity. To test the hypothesis that PDEs act locally within signalosomes to influence cell function, the group used peptide arrays to identify the peptide motifs that allowed PDEs to interact with other proteins in their signalosome. They then designed cell-permeable disruptor peptides with which they displaced the endogenous PDEs from their complexes. Maurice explains: “Using these disruptor peptides, or small molecules which mimic their effects, we predict that it should be possible to selectively target cyclic nucleotide-mediated events specific to individual signalosomes”.

The lab has identified multiple signalling complexes that pair cAMP or cGMP effector proteins – the protein kinases A and G, and the exchange protein EPAC – alongside PDEs to form functional units in cells that then monitor cyclic nucleotide signalling. Using fluorescent microscopy and live cell imaging, they examine these complexes in their specific subcellular locations and how they dynamically regulate increases in cAMP or cGMP. Through these means, the group has confirmed that signalling occurs spatially and temporally within cells, and that the location of the signalling enzymes is fundamentally important to specific cell actions.

TRANSLATING RESEARCH INTO TREATMENT

PDE enzymes are known to present good targets for drug development, especially those of the PDE5 family, for which drugs have been produced to treat conditions such as erectile dysfunction and pulmonary hypertension. Also, PDE4 inhibitors have recently been licensed for treating chronic obstructive pulmonary disease. These drugs are, however, known to have side effects such as diarrhoea, weight loss and pancreatitis. By targeting the specific interactions between PDEs and their partner proteins within individual signalosomes, Maurice is hoping to improve drug strategies for diseases such as atherosclerosis without incurring undesirable side effects: “Should our approach prove robust, we predict that it would reduce the off-target effects associated with seeking out the entire cellular pool of a given PDE, as is currently the case using traditionally designed agents which target the active site,” he asserts. The approach holds promise for other diseases and Maurice anticipates that proof of concept will lead to much interest from the pharmaceutical industry: “We are currently refining and testing our approach in house”. We hope that our studies will evolve to include active collaborations with pharmaceutical companies – so our research may turn out to be highly translational”.

INTeligence

MAURICE LAB

OBJECTIVES

To understand the molecular basis of cyclic nucleotide-mediated effects in vascular tissues. More specifically, the laboratory studies the enzymes that synthesise cAMP and cGMP (adenylyl cyclase and guanylyl cyclase) and those which catabolically inactivate these two second messengers (cyclic nucleotide phosphodiesterases) using multiple parallel and complementary therapies.

KEY COLLABORATORS

Dr Miles Houslay; Dr George Baillie, University of Glasgow, UK • Dr Manuela Zaccolo, University of Oxford, UK

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DR DONALD MAURICE obtained a BSc in Biochemistry from Carleton University in Ottawa and a PhD in Medical Sciences for studies conducted with Dr Richard Haslam at McMaster University in Hamilton, Canada. After graduation, he carried out Medical Research Council of Canada-funded postdoctoral studies in the laboratory of Dr T Kendal Harden at the University of North Carolina, Chapel Hill, USA. Maurice joined Queen’s University in 1994 as Assistant Professor in the Departments of Pathology & Molecular Medicine and Pharmacology & Toxicology. Maurice’s work has resulted in the publication of 50 scientific manuscripts. He is a member of the Editorial Board of Molecular Pharmacology, and a grant reviewer on the Cardiovascular Sciences Committee of the Canadian Institutes for Health Research of Canada Open Suite of Programs.