GPCRs in cardiovascular pathologies

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A central problem in the management of patients with cardiovascular and related diseases is the often complex or unknown disease etiology. For instance, physicians still treat hypertensive cardiac disease empirically choosing from many antihypertensive medications until one drug or a combination of drugs is identified, which effectively reduces blood pressure in the patient (R.P. Lifton *et al.*, *Cell*. 2001;104:545–556). However, a significant blood pressure reduction can often be insufficient to prevent or induce regression of co-morbidities (e.g. diabetes or obesity) or their consequences, including cardiac and renal dysfunction, atherosclerosis or cerebrovascular events (I. Biaggioni, *Hypertension*. 2008;51:168–171).

It can be argued that the co-morbidities such as diabetes and obesity are *de facto* causative of cardiovascular disease. Various co-morbidities as well as genetic and environmental factors (e.g. work-place stress) can all have similar cardiovascular consequences, suggesting that they all activate common largely overlapping signaling circuits in the cardiovascular system. Arguably, similar reasoning could be made for the development of pulmonary arterial hypertension (PAH), cardiovascular inflammation, cardiac arrhythmias and cardiovascular abnormalities that occur during pregnancy. Evidence is mounting that susceptibility to cardiovascular and metabolic disease in adulthood is largely determined by perinatal conditions. Would it then be possible to interrupt or counter the course of the program that predisposes us to cardio-metabolic disease? The identification of common mediators downstream of diverse causative factors would enable a better understanding of cardiovascular related pathologies as well as enhance our ability to effectively treat them. Conceivably, targeting common signaling molecules that are predominantly activated under pathological conditions should enable us to treat cardiovascular diseases with complex or unknown etiology.

Decades of intense research identified G-protein-coupled receptors (GPCRs) as key mediators downstream of multiple causative factors, including diabetes, obesity, environmental stressors and genetic factors, which increase susceptibility to cardiovascular disease development. GPCRs represent a diverse family of cell surface receptors with more than 800 members, accounting for ~3–4% of the total genes encoded in the human genome. GPCR dysfunction contributes to the most prevalent human cardiovascular diseases and GPCRs are particularly amenable to pharmacological modulation with low molecular weight compounds. While about 30% of the top-selling 500 prescription drugs act, directly or

indirectly, on GPCRs, the currently available GPCR agonists/antagonists target approximately 20 receptors (Pettipher *et al.*, *Curr. Opin. Pharmacol.* 2005;5:412–417).

A growing body of evidence indicates that the complexity of the GPCR signalosome cannot be simply explained by the ‘on’ and ‘off’ states of the receptor in the presence of an agonist or antagonist. For instance, signaling from multiple GPCRs may converge to the same downstream targets, including the GRK/arrestin machinery or EGF receptor transactivation; activation of one GPCR may block signaling through another GPCR; biased ligand signaling or partial antagonists may elicit only desired biological activities associated with GPCR activation and pleiotropic GPCRs, such as the formyl peptide receptors integrate diverse signals to mediate opposing biological responses. Better understanding of these mechanisms has great potential to identify novel specific targets for pharmacological intervention to counter cardiovascular diseases. This Special Issue of *Drug Discovery Today: Disease Models* explores recent advances in the GPCR signalosome during pathological conditions, including cardiac arrhythmias, systemic and pulmonary artery hypertension, heart failure, pre-eclampsia, inflammation, pain and early programming of the metabolic syndrome.

Metabolic syndrome is characterized by the co-occurrence of obesity, dyslipidemia, impaired glucose homeostasis and increased blood pressure, thus predisposing to major cardiovascular and metabolic complications. It is now recognized that exposure to certain stressors during pregnancy or early childhood, a phenomenon also known as early programming, predisposes to development of metabolic and cardiovascular diseases later in life. Christian F. Rueda-Clausen and Sandra T. Davidge discuss the potential role of GPCRs in the imprinting of the metabolic syndrome. The authors review evidence suggesting that GPCR-dependent mechanisms could also play a fundamental role in the early programming of metabolic syndrome. They also assess the phenotypical changes observed in animal and clinical models of fetal programming, discuss how the fetal programming mechanisms and GPCRs may contribute to the development of the metabolic syndrome and explore the therapeutic potential of targeting GPCRs for the prevention and/or treatment of the metabolic syndrome.

The ‘On–Off’ states of ligand-induced signaling have been at the core of the traditional view of agonist-induced GPCR activation. This notion has now been challenged as agonists with different binding affinities for their receptor can induce various degrees of receptor conformational changes to engage different intracellular signaling pathways. Stephen L. Belmonte and Burns C. Blaxall review the emerging area of biased ligand signaling. They argue that the classical notion of GPCR signaling, which posited that receptors shuttle between ‘on’ and ‘off’ states in the presence or absence of agonist, respectively might be inadequate to explain the growing complexity of the GPCR signalosome, which includes receptor desensitization and downregulation, allosteric modulation, partial agonism and ligand bias. Two of the most important cardiovascular GPCRs, adrenergic receptors and angiotensin II type 1 receptors are among the best described examples of biased ligand signaling, which may be exploited to produce novel benefit from established targets. Belmonte and Blaxall also discuss current GPCR pharmacotherapy in cardiovascular diseases and highlight promising avenues for future drug development.

Exploring further the complexity of the GPCR signalosome, Scott P. Heximer discusses a class of intracellular regulators of GPCR pathways, the regulators of G-protein signaling or RGS. These inhibitory proteins belong to a protein superfamily that enhances the GTPase activity of the $G\alpha$ subunit of G-proteins. Heximer reviews emerging evidence indicating an important role for RGS proteins during physiologic and pathophysiological conditions in cardiovascular tissues and highlights how altering intracellular levels and/or function of RGS proteins may provide unique therapeutic opportunities for the treatment and prevention of cardiovascular disease.

The GPCR signalosome involves positive forward and negative feedback circuits and cross-talks between GPCRs and effector pathways, for instance growth factor receptors and ion channels. Exaggerated growth factor receptor signaling underlies the hypertrophy and fibrosis and is mediated, at least in part, by metalloproteinases. Dysregulation of ion channels is a likely cause of cardiac arrhythmias, which trigger sudden cardiac death. In the review by Peter Zylbergold and Terence E. Hébert, a family of effectors of GPCR signaling pathways, the G-protein-modulated inwardly rectifying potassium channels (Kir3, GIRK) are brought into focus in light of their role in the development of cardiac arrhythmias as well as in the stabilization of resting membrane potential in both excitable and non-excitable cells. In particular, Kir3 channel complexes emerged as important contributors to the development of atrial fibrillation and long QT syndrome in patients. Zylbergold and Hébert argue that while GPCRs or Kir3 channels themselves might be interesting therapeutic targets, development of drugs that target complexes formed by these molecules might result in more selective and specific approach to treat certain types of cardiac arrhythmias. This raises the intriguing question whether targeting specific GPCR/effector complexes (such as GPCR/Kir 3 complexes) might be more relevant for drug discovery than the traditional approach of targeting the ligand binding sites of GPCRs.

Identifying the mechanisms linking the metabolic syndrome and environmental stressors to cardiovascular disease is a major challenge. Xiang Wang, Ana-Maria Bosonea and Carlos Fernandez-Patron discuss that many primary causative factors of hypertensive cardiac disease, including environmental stressors, genetic predisposition, obesity and diabetes, act through triggering systemic production of common GPCR agonists. Excessive production of these GPCR agonists would then transactivate common signaling circuits, for instance matrix metalloproteinases, which relay and amplify agonist signaling. Wang *et al.* propose a global therapeutic concept for treatment of hypertensive cardiac disease with complex or unknown etiology by targeting common mediators of multiple GPCRs such as metalloproteinases, their activators and effectors.

While GPCR agonist-induced systemic arterial hypertension has little influence on the pulmonary circulation, excessive constriction of the pulmonary artery leads to PAH, which is often associated with hypertrophy and dysfunction of the right ventricle. Roxane Paulin and Evangelos Michelakis review how dysregulated GPCR signaling contributes to the development and progression of PAH and in particular to the proliferative phenotype of pulmonary artery smooth muscle cells. While ET-1 receptor A blockers, prostacyclin analogues and phosphodiesterase type 5 inhibitors are the currently approved therapy for PAH treatment, a major challenge is that while vascular remodeling is restricted to the

pulmonary circulation, the signal(s) that triggered PAH (e.g. hypoxia or inflammation or exposure to anorexic agents) could also be present within the body. Paulin and Michelakis propose novel GPCR circuits that could potentially be involved in PAH.

Pre-eclampsia, an elusive cardiovascular condition characterized by severe hypertension, edema and renal dysfunction, occurs during pregnancy and is a potentially life-threatening condition. Jonathan T. McGuane and Kirk P. Conrad review the role GPCRs, the soluble forms of the angiogenic membrane receptor tyrosine kinase Flt1 and the transforming growth factor β co-receptor endoglin in pre-eclampsia. They discuss several promising GPCRs, including calcitonin receptor-like receptor/receptor activity modifying protein 1 complexes, the angiotensin AT2 and Mas receptors and the relaxin receptor RXFP1 as potential targets for the prevention and/or treatment of pre-eclampsia.

Excessive or dysregulated intravascular inflammation, an initially protective immune response against invading pathogens and tissue injury, underlies many pathological conditions, including cardiovascular diseases. János G. Filep and Driss El Kebir review the role of protein and lipid ligand-activated GPCRs in the amplification as well as in the resolution of the inflammatory response. They describe a concept of targeting common downstream GPCR regulators to limit the extent of the initial inflammatory response. This strategy echoes those proposed for the treatment of hypertensive cardiac disease of complex etiology, cardiac hypertrophy and fibrosis in other chapters of this Special Issue. Filep and El Kebir also discuss the emerging role of formyl peptide receptors and their numerous protein and lipid ligands in orchestrating timely resolution of inflammation, and suggest that this process might be impaired in patients with cardiovascular diseases.

Perception of pain is often the first and alarming symptom of cardiovascular diseases. Pedro D'Orleans-Juste and colleagues review the contribution of bradykinin and endothelin-1 to pain perception. Both bradykinin and endothelin-1 have been implicated in modulating nociceptive response and hyperalgesia associated with angina pectoris, angioedema or peripheral vascular diseases. A direct cross-talk between these GPCR agonists may occur via the TLR2/CXCR2/brady-kinin B2 complex as reported in Chagas' cardiomyopathy. While pain control is usually not a primary endpoint in clinical trials, endothelin-1 and/or bradykinin receptor blockade may help to alleviate pain and therefore would probably contribute to a better pain management in patients with cardiovascular disease.

In conclusion, multiple factors, environmental, genetic and life style related, can result in dysregulated GPCR signaling in the cardiovascular system. The common consequence of abnormal GPCR signaling is the development of diverse cardiovascular pathologies, including hypertrophic and fibrotic remodeling of left and right cardiac ventricles and remodeling of arteries, which occur in association with systemic and pulmonary hypertension. In addition, dysregulated GPCR signaling can trigger vascular inflammation, atrial fibrillation, cardiovascular pain perception and heart failure. An emerging and clinically relevant issue is that considering about 400 GPCRs known to date, which may affect cardiovascular functions, and the high number of agonists and antagonists available for some GPCRs, it might be overly simplistic and probably inefficient to aspire to treat complex diseases by pharmacologically targeting one or only a few individual GPCRs. Thus,

further identification of common signaling molecules positioned downstream of multiple GPCRs that are predominantly activated under certain pathological conditions may hold promise for development of more efficient strategies for the treatment of cardiovascular diseases, in particular those with complex or unknown etiology.

Biographies

Carlos Fernandez-Patron started his career as an independent investigator in 2001 in the Department of Biochemistry, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada. From 1983 to 1989, he studied Physics, at the University of Technology (Dresden, Germany), receiving his MSc degree in Low Temperature Physics.

In 1990, while a PhD student, he co-invented a 'reverse stain' method for detecting biomacromolecules such as proteins, DNA, RNA and LPS using heavy metal ions. This 'reverse stain' method is now marketed worldwide. He obtained his PhD degree in 1995 with work in the area now termed 'proteomics' at the Center for Genetic Engineering and Biotechnology (Havana, Cuba).

From 1992 to 1997 while still conducting PhD and post-doctoral studies, he trained at The Wellcome Research Labs in Beckenham (UK, under Prof. Sir Salvador Moncada), Hospital Ramon y Cajal (Madrid, Spain with Dr. Enrique Mendez), at the Zentrum fuer Molekulare Biologie (Heidelberg, Germany, under supervision of Dr. Rainer Frank) and at the European Molecular Biology Laboratory (Heidelberg, Germany, in the Protein and Peptide Group led by Prof. Matthias Mann). From 1997 to 2001, he conducted a post-doctoral fellowship in the Department of OB/GYN, University of Alberta (Edmonton, Canada, under supervision of Drs. Sandra Davidge and Marek Radomski).

He has received fellowships and scholarships from the Deutscher Akademischer Austauschdienst (DAAD, Germany), Molly Towel Foundation for Perinatal Research, Alberta Heritage Foundation for Medical Research, Canadian Hypertension Society, Heart and Stroke Foundation of Canada and Canadian Institutes of Health Research.

The long-term goal of his research is to develop global therapeutic strategies applicable to human hypertension with complex or unknown etiology. Specifically, Dr. Fernandez-Patron is examining the therapeutic potential of targeting signaling molecules positioned downstream of vasoactive GPCRs and that are predominantly activated under pathological conditions. He has proposed that among such molecules are metalloproteinases, metalloproteinase activators and metalloproteinase substrates. His research has been supported by Heart and Stroke Foundation of Canada, Canadian Institutes of Health

Research and Natural Sciences and Engineering Council of Canada (NSERC) operating grants as well as studentships from NSERC and Alberta Innovates Health Solutions.

János G. Filep is a Professor of Pathology and Cell Biology at the University of Montreal and Director of the Laboratory of Vascular Immunity/Inflammation at the Research Center, Maisonneuve-Rosemont Hospital. He received his MD degree from Semmelweis University Medical School. After post-doctoral training at the Department of Clinical Pharmacology of Hannover Medical College, Hannover, Germany and the Department of Pharmacology, University of Sherbrooke, Sherbrooke, QC, Canada, he joined the Faculty of Medicine at the University of Montreal in 1992. His main research interest is how interactions of the innate immune system with blood vessels contribute to development of cardiovascular diseases, in particular acute coronary artery disease and septic shock. He has received Fellowship awards from the Boehringer Ingelheim Foundation, Stuttgart, Germany and the Medical Research Council of Canada, a Senior Scholarship award from the FRSQ (Quebec Health Research Funds), Canada. He has received the Science and Peace Medal from the Albert Schweitzer International University, Madrid, Spain and Gold Medal for Canada from the American Biographical Institute, N.Y. His current research interests have focused on how modulation of neutrophil apoptosis affect outcome of the acute inflammatory response applied to cardiovascular and pulmonary pathophysiology. His team investigates how beta-2 integrins and pleiotropic formyl peptide receptors on leukocytes integrate multiple signals to mediate opposing cellular responses. Recently, the team showed the beneficial anti-inflammatory and pro-resolution actions of therapeutic induction of neutrophil apoptosis with aspirin-triggered lipoxins. His research has been supported by the Canadian Institutes of Health Research, Heart and Stroke Foundation of Canada and Natural Sciences and Engineering Council of Canada.