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Minireview

GPCR responses in vascular smooth muscle can occur predominantly through dual transactivation of kinase receptors and not classical $G_{\alpha q}$ protein signalling pathways

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ABSTRACT

GPCR signalling is well known to proceed through several linear pathways involving activation of G proteins and their downstream signalling pathways such as activation of phospholipase C. In addition, GPCRs signal via transactivation of Protein Tyrosine Kinase receptors such as that for Epidermal Growth Factor (EGF) and Platelet-Derived Growth Factor (PDGF) where GPCR agonists mediate increase levels of phosphorylated Erk (pErk) the immediate downstream product of the activation of EGF receptor. It has recently been shown that this paradigm can be extended to include the GPCR transactivation of a Protein Serine/Threonine Kinase receptor, specifically the Transforming Growth Factor β Type I receptor (also known as Alk V) (TβRI) in which case GPCR activation leads to the formation of carboxy terminal polyphosphorylated Smad2 (phosphoSmad2) being the immediate downstream product of the activation of TβRI. Growth factor and hormone regulation of proteoglycan synthesis in vascular smooth muscle cells represent one component of an *in vitro* model of atherosclerosis because modified proteoglycans show enhanced binding to lipoproteins as the initiating step in atherosclerosis. In the example of proteoglycan synthesis stimulated by GPCR agonists such as thrombin and endothelin-1, the transactivation pathways for the EGF receptor and TβRI are both active and together account for essentially all of the response to the GPCRs. In contrast, signalling downstream of GPCRs such as increased inositol 1,4,5 trisphosphate (IP_3) and intracellular calcium do not have any effect on GPCR stimulated proteoglycan synthesis. These data lead to the conclusion that dual transactivation pathways for protein tyrosine and serine/threonine kinase receptors may play a far greater role in GPCR signalling than currently recognised.

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Introduction

Guanine Nucleotide Binding Protein Coupled Receptors (GPCRs) constitute the largest class of cell surface receptors and their genes account for a remarkable 5% of the human genome (McCudden et

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al., 2005; Morris and Malbon, 1999). Drugs targeting GPCRs are the largest class of therapeutic agents for diseases including cardiovascular disease, cancer and asthma (McCudden et al., 2005; Morris and Malbon, 1999). The current paradigm of GPCR signalling covers three major pathways: firstly, the classic G protein coupled pathway in which ligand engagement causes G protein binding to its receptor and G_{α} complexes then regulates the activity of a large number of effector molecules, secondly, the β-arrestin scaffold pathway which also leads to activation of multiple downstream signalling cascades and thirdly, the transactivation of Protein Tyrosine Kinase receptors (Daub et al., 1997, 1996; Prenzel et al., 1999). The GPCR transactivation of Protein Tyrosine Kinase (PTK) receptors has been recently reviewed (Liebmann, 2011; Liebmann and Bohmer, 2000). In this final manifestation, this phenomenon of GPCR to PTK receptor transactivation was first described in Ullrich's laboratory in 1996 and has subsequently been the subject of almost two hundred reports (Daub et al., 1996). These reports have sought to understand the diversity of the GPCR and PTK receptor partners involved and the signalling pathways and hence mechanism(s) of transactivation. GPCR transactivation of PTK receptors has been associated with multiple pathologies including hypertension and hyperglycaemia in animal models of Type 2 diabetes (Nagareddy et al., 2010).

We have recently reported evidence that GPCRs can also transactivate protein serine/threonine kinase (PS/TK) receptors (Burch et al., 2010a, 2013; Little et al., 2011, 2010; Tang et al., 2010). It is noted that several others had described this interaction without specifically applying the concept of this being a transactivation pathway to describe the interaction and response (Liu et al., 2009; Xu et al., 2009). We focus on proteoglycan synthesis in VSM as one component (the other is LDL binding) of an *in vitro* model of atherosclerosis (Ballinger et al., 2004; Little et al., 2007, 2008). In the context of proteoglycan synthesis and atherosclerosis the transactivation pathways are emerging as the major pathways through which GPCR agonists control the multiple facets of proteoglycan, specifically biglycan expression and glycosaminoglycan (GAG) elongation, synthesis in these cells (Burch et al., 2010a; Little et al., 2010).

In some circumstances the transactivation of PTK receptor pathways has to some extent been considered almost an epiphenomenon and not a major contributor to GPCR signalling (Hill, 2006). However, our work is suggesting that transactivation pathways may indeed be very important and further, that signalling of some responses such as proteoglycan synthesis may be entirely dependent upon transactivation pathways and not classical $G_{\alpha q}$ protein pathways. In this review we consider the possibility that transactivation of kinase receptors may mediate some GPCR signalling without involvement of traditional downstream mediators such as calcium ions and IP_3 (Lefkowitz, 2007b; Liebmann and Bohmer, 2000).

Therapeutic context of proteoglycan synthesis for the study of GPCR signalling

The medical context of our work on signalling pathways that control proteoglycan and GAG synthesis is that atherosclerosis and its clinical sequelae of plaque rupture (Falk, 1989) remain one of the major contributors to morbidity and premature mortality (Haffner and Cassells, 2003; Haffner et al., 1998). Atherosclerosis-related cardiovascular disease is still the major cause of premature mortality notwithstanding the extended availability of the highly efficacious lipid-lowering statin drugs (Anon., 1994). The current gold standard for the medical therapy to prevent and retard atherosclerosis is the HMGCoA reductase inhibitors, statins, such as atorvastatin and rosuvastatin (Anon., 1994; Ehrenstein et al., 2005). Accordingly, it is proposed that the desirable therapeutic agent would be a statin combined with an agent inhibiting growth factor and hormone mediated signalling pathways stimulating proteoglycan synthesis such that the statin would lower plasma cholesterol and the proteoglycan inhibitor would render the vessel wall "less sticky" for the atherogenic lipids that can accumulate in the wall (Little et al., 2007).

Human atherosclerosis in susceptible vessels such as coronary and carotid arteries commences with the penetration, binding and retention of atherogenic lipoproteins by modified proteoglycans (Nakashima et al., 2007; O'Brien et al., 1998). Many cardiovascular and diabetes drugs have pleiotropic actions on proteoglycan synthesis by VSMCs (de Dios et al., 2003, 2007; Nigro et al., 2004, 2002; Tannock et al., 2002). The major structural modifications to proteoglycans responsible for increased lipid retention in the vessel wall are an elongation of chondroitin sulphate/dermatan sulphate (CS/DS) GAG chains on for example, biglycan, a response which we term hyperelongation (Little et al., 2008); other structural changes such as an alteration in the GAG chain sulfation pattern contribute to increased lipid binding but this has been less well studied at this time (Ballinger et al., 2004). Both hyperelongation and modified sulfation increase the binding between proteoglycans and lipoproteins (Ballinger et al., 2004).

Vascular smooth muscle cells (VSMCs) are the major source of proteoglycans in the vessel wall (Wight, 1995). VSMCs express numerous GPCRs and the actions of their agonists underlie much cardiovascular disease. For example, antagonists of angiotensin II system are amongst the most efficacious agents in cardiovascular therapeutics (Dahlof et al., 2002; Parving and Rossing, 2001). GPCR agonists including angiotensin II, endothelin-1 (ET-1) and thrombin also act on VSMCs to modify proteoglycan synthesis in a manner that increases the binding to lipoproteins (Ballinger et al., 2009; Figueroa and Vijayagopal, 2002; Ivey and Little, 2008; Shimizu-Hirota et al., 2001). There has been some work to define the downstream signalling pathways through which GPCR agonists stimulate proteoglycan synthesis (Ballinger et al., 2009; Figueroa and Vijayagopal, 2002; Ivey and Little, 2008) and this is of considerable interest because identifying a common pathway for the regulation of proteoglycan synthesis has been proposed as a target for the prevention of atherosclerosis (Ballinger et al., 2004; Little et al., 2007). Thus, as above, GPCR signalling and its application to the synthesis of proteoglycans by VSMCs are the subject of this review.

Classical $G_{\alpha q}$ protein pathways

GPCRs have no intrinsic enzymatic capabilities (Lefkowitz, 2007a,b) in contrast for example to protein kinase receptors which have intrinsic kinase activity which mediates autophosphorylation of the receptors and phosphorylation of immediate downstream targets such as Erk and Smad transcription factors (Derynck and Zhang, 2003; Heldin, 2004; Heldin et al., 1997; Massague, 2003; Ross, 1987). GPCRs are coupled to G proteins (G_{α}, G_{β} and G_{γ}) which act as effectors of GPCR signalling. GPCR agonists such as thrombin, ET-1 and angiotensin II engage their respective receptors Protease Activated Receptor (PAR)-1, $ET_{A\ and\ B}$ and $AT_{1\ and\ 2}$ leading to a conformational change in the receptor causing activation of the C-terminally bound G-proteins which dissociate and propagate signals via secondary messengers (Hendriks-Balk et al., 2008; Lefkowitz, 2007b; McCudden et al., 2005). The $G\alpha_q$ protein leads to signal propagation by activating phospholipase C (PLC). PLC then hydrolytically cleaves a membrane-bound phospholipid, phosphoinositol 4,5-bisphosphate (PIP2). PIP2 is cleaved into DAG and IP_3. DAG remains bound to the membrane whereas highly hydrophilic IP_3 is released as a soluble messenger into the cytosol. IP_3 diffuses through the cytosol to bind to IP_3 receptors in the smooth endoplasmic reticulum (ER). As the concentration of calcium ions in the endoplasmic reticulum is extremely highly relative to the free cytosolic concentration there is a rapid and massive release of calcium ions into the cytosol and entry via calcium channels and the concentration of calcium in for example a VSMC can rise from less than one hundred nanomolar to above one micromolar in seconds (Neylon et al., 1992). A multitude of cellular processes are sensitive to calcium ions, such as