G protein-coupled receptors in cardiac biology: old and new receptors

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Abstract G protein-coupled receptors (GPCRs) are seven-transmembrane-spanning proteins that mediate cellular and physiological responses. They are critical for cardiovascular function and are targeted for the treatment of hypertension and heart failure. Nevertheless, current therapies only target a small fraction of the cardiac GPCR repertoire, indicating that there are many opportunities to investigate unappreciated aspects of heart biology. Here, we offer an update on the contemporary view of GPCRs and the complexities of their signalling, and review the roles of the ‘classical’ GPCRs in cardiovascular physiology and disease. We then provide insights into other GPCRs that have been less extensively studied in the heart, including orphan, odorant and taste receptors. We contend that these novel cardiac GPCRs contribute to heart function in health and disease and thereby offer exciting opportunities to therapeutically modulate heart function.

Keywords G protein-coupled receptor · Heart · Cardiovascular disease · Orphan receptor · Odorant receptor · Taste receptor

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Introduction

G protein-coupled receptors (GPCRs) are specialised integral membrane proteins with a seven-transmembrane α -helix structure that represent the largest receptor superfamily in the human genome, comprising more than 800 protein-coding genes (Lagerstrom and Schiöth 2008). GPCRs recognise and bind an array of sensory inputs and endogenous ligands including photons, ions, bioamines, lipids, carbohydrates, peptides and proteins, as well as a diverse range of volatile compounds. Ligand-induced activation of GPCRs converts extracellular stimuli into intracellular signals to mediate diverse cellular and physiological responses. GPCRs have been extensively studied in the heart and cardiovascular system, where activation of these receptors has profound homeostatic and regulatory effects. Mutations and modifications of GPCRs, G proteins and their regulatory partners are associated with physiological dysfunction and disease, as well as with variable therapeutic outcomes. The importance of these receptors is reflected in the fact that over one-third of drugs on the market target GPCRs (Rask-Andersen et al. 2011).

GPCR-modulating therapeutics are particularly successful in the treatment of cardiovascular disease (CVD), including hypertension, arrhythmias and heart failure (DeWire and Violin 2011). Nonetheless, as CVD remains a leading cause of morbidity and mortality (Go et al. 2014), there remains a significant and as yet unmet need for improved cardiovascular therapeutics. In this article, we provide an overview of GPCRs and their pleiotropic signalling pathways and discuss recent developments in the field. We then highlight the receptor systems that have been classically targeted in the heart and cardiovascular system and provide insights into novel GPCRs that may contribute to the regulation of cardiac function in health and disease.

Recent advances in GPCR physiology and pleiotropic signalling

The GPCR superfamily is a phylogenetically diverse group of membrane proteins that have been functionally implicated in all organ systems. The classical model of GPCR signal transduction holds that ligand binding induces a dynamic conformational change in the receptor that favours association with the $G\alpha\beta\gamma$ -heterotrimeric complex and promotes the exchange of guanosine diphosphate for guanosine triphosphate on the G protein α -subunit (Rodbell 1995). These activated G proteins can engage and modulate numerous downstream effector enzymes and ion channels to initiate diverse cellular responses (Hamm 1998).

In recent years, in keeping with progress in GPCR research, the notion of a simple ligand–receptor–G protein–effector enzyme signalling cascade has been replaced with increasingly complex pharmacological models (reviewed in Kenakin and Miller 2010; Lagerstrom and Schiöth 2008; Luttrell 2008). Closely tied to the development of molecular and physiological assays and techniques to probe receptor function, the nuances and intricacies of GPCR signalling and their implications for physiology and disease are emerging (Fig. 1a).

Most importantly, it is now recognised that GPCRs couple to a wide variety of intracellular proteins, both dependently and independently of G proteins. This has led to the alternative all-encompassing nomenclature of seven-transmembrane (7TM) receptors (rather than GPCRs). According to the traditional view, signalling complexity is provided by the differential combinatorial assembly of the different heterotrimeric G proteins (including the 16 $G\alpha$, 5 β and 12 γ subunits) and the pleiotropic coupling of one receptor to multiple types of G proteins and corresponding signalling pathways (Gudermann et al. 1996; Luttrell 2008). We now understand that receptors can be differentially expressed and alternatively spliced in different cell types and tissues (Einstein et al. 2008), whereas increased specificity in GPCR signalling is achieved by coupling to multiple isoforms of effectors and by their different sub-cellular localisation (Insel 2003; Ostrom and Insel 2004). Moreover, GPCRs are capable of interacting to form dimers or oligomers, which may have important signalling and functional consequences (Albizu et al. 2010; Milligan 2007).

GPCRs also bind to numerous other cellular proteins that modulate receptor function or recruit additional signalling pathways. These include receptor activity-modifying proteins (RAMPs) (Parameswaran and Spielman 2006), regulators of G protein signalling (RGS) proteins (Brinks and Eckhart 2010), PDZ motif-containing proteins (Harris et al. 2008; Milligan and White 2001), GPCR kinases (GRKs) (Gurevich et al. 2012) and arrestins (DeWire et al. 2007). For certain GPCRs, including the angiotensin type 1 receptor, ligand activation can lead to crosstalk between distinct signal

transduction pathways, such as the receptor tyrosine kinases (RTKs), leading to even more diverse cellular responses (George et al. 2010; Luttrell 2008). Ultimately, the fine-tuning and regulation of GPCR signalling is mediated via various post-translational modifications, including phosphorylation, acetylation, palmitoylation, ubiquitination and myristoylation (Millar and Newton 2010).

In addition, agonist ligands do not uniformly activate all cellular signalling pathways linked to a given GPCR, a phenomenon known as ligand or agonist bias (Kenakin and Christopoulos 2013). This discovery has changed the way potential novel therapeutic leads are developed in high-throughput screens and reshaped the way that the pharmacological concept of efficacy is considered. According to the traditional view, agonist binding stabilises a single ‘active’ state of a GPCR, leading to a response that could vary in magnitude depending on the binding properties of that ligand. It is now widely accepted that receptors instead form multiple states that facilitate pleiotropic coupling to other intracellular proteins (Kobilka and Deupi 2007; Vaidehi and Kenakin 2010). As our understanding (and measurement) of multiple receptor states and the broader agonist-mediated signalling networks has progressed, it has become clear that there are important differences in ligand-driven responses at a particular receptor. This ligand-mediated signalling bias has also been called ‘functional selectivity’, whereby one ligand selectively stimulates one signal whereas a different ligand can selectively elicit a different pattern of responses via the same receptor (Fig. 1b) (DeWire and Violin 2011).

The promise of biased agonists in relation to therapeutics is that these compounds offer the potential to stimulate a desired receptor response without activating ‘on-target’ adverse or less desirable signalling effects (DeWire and Violin 2011). Although accumulating evidence from *in vitro* studies suggests that many GPCRs display biased agonism, the extent of translation of this phenomenon *in vivo* remains unclear (reviewed extensively by Kenakin and Christopoulos 2013). Nonetheless, biased signalling at a number of GPCRs may be relevant in the treatment of disease, including depression (targeting α_{2A}) (Cottingham et al. 2011), schizophrenia (5-HT_{2A}) (Schmid et al. 2008), osteoporosis (parathyroid hormone receptors) (Gesty-Palmer et al. 2009), asthma (Walker et al. 2011), as well as CVD (discussed in detail in following sections). Three compounds that display biased signalling at GPCRs are currently being studied in clinical trials for the treatment of heart failure and acute and moderate pain (DeWire et al. 2013; Violin et al. 2010).

Similarly, the understanding of GPCRs as conformationally flexible proteins has been accompanied by an increased interest in allosteric ligands. These ligands modulate GPCR signalling by binding to sites topographically distinct from the orthosteric (or endogenous) ligand binding site (Melancon et al. 2012; Wootten et al. 2013). GPCR-

Fig. 1 Complexities of G protein-coupled receptor (GPCR) signalling. **a** The contemporary view of seven-transmembrane α -helix (7TM) receptor signalling extends far beyond the G protein, with receptor function influenced by an array of interacting proteins and processes. **b** Biased signalling: two agonists can bind to the same receptor to elicit different conformations/receptor states and thus favour different signalling responses. **c** Allosteric interactions: receptor function can be influenced by interactions with other proteins [such as receptor activity-modifying proteins (RAMPs) and G protein-coupled receptor kinases (GRKs)] or binding of allosteric ligands. *RTKs* Receptor tyrosine kinases

interacting proteins can also act as allosteric modulators of receptor function. In this manner, allosteric ligand/protein interactions lead to conformational changes in the receptor protein that can affect the orthosteric site and/or effector protein coupling sites (Fig. 1c) (Kenakin and Miller 2010). As has been discussed in several comprehensive reviews, allosteric ligands provide novel opportunities to modulate GPCR function that cannot be achieved by ligands that bind to the orthosteric site, including a saturability of response that may limit overdose and potentially increase GPCR subtype selectivity (Kenakin and Miller 2010; May et al. 2007; Melancon et al. 2012; Wootten et al. 2013). Indeed, there are a number of GPCRs for which allosteric modulators have shown therapeutic promise, such as the prostaglandin F receptor (Goupil et al. 2010), muscarinic M_4 (Chan et al. 2008) and glucagon-like peptide-1 receptor (Koole et al. 2010). To date, allosteric modulators have been approved for the treatment of hyperparathyroidism (positive modulator targeting the calcium sensing receptor) (Block et al. 2004) and human immunodeficiency virus type 1 (negative allosteric modulator of CCR5) (Fatkenheuer et al. 2005).

In recent years, there have been dramatic advances in the application of structural biology techniques to GPCRs, resulting in the elucidation of numerous high-resolution structures of *Rhodopsin* family (class A) GPCRs (Audet and Bouvier 2012; Katritch et al. 2013). Structures for many of the prototypical GPCRs have been published, including those of the β_1 - and β_2 -adrenoceptors receptor, muscarinic, dopamine, serotonin, chemokine, sphingolipid, adenosine and opioid GPCR subtypes (Katritch et al. 2013; available at <http://gpcr.scripps.edu/>). These structures were determined when bound to various inverse agonists or neutral antagonists (inactive conformations), or less frequently with agonist ligands (active conformations). A particularly important landmark in GPCR biology was achieved with the determination of the active-state ternary complex of β_2 -AR in complex with the heterotrimeric G protein (Rasmussen et al. 2011). Similarly, the recent crystallisation of active β -arrestin in complex with a peptide derived from the human V2 vasopressin receptor has shed light on the potential molecular mechanism for the interaction between GPCRs and signalling and regulatory proteins (Shukla et al. 2013). Kruse et al. (2013)

recently determined the structure of agonist bound M_2 -receptor in combination with a positive allosteric modulator, paving the way for obtaining drug selectivity and therapeutic targets. Taken together, the multiple structures illustrate the ways in which the 7TM architecture can accommodate diverse ligands and how GPCRs can exist in multiple conformational states, and has provided a consensus view on the processes that underpin ligand binding and receptor activation (Venkatakrishnan et al. 2013). The consistent refining of high-throughput pipelines is resulting in the elucidation of additional high-resolution GPCR structures, including receptors from the *Glutamate* (Wu et al. 2014), *Secretin* (Hollenstein et al. 2013; Siu et al. 2013) and *Frizzled/TAS2* families (Wang et al. 2013, 2014), and undoubtedly more will follow. In combination with novel computational approaches and molecular dynamics simulations (Dror et al. 2011a, b, 2013; Nygaard et al. 2013), researchers in the field can continue to gain new structural insights in GPCR activation and signalling. It is hoped that these insights will lead to structure-based rational drug design (Shoichet and Kobilka 2012).

Thus, spurred on by the tractability of these receptors as therapeutic targets, GPCRs are now viewed as complex, dynamic proteins capable of binding to an array of partners to elicit diverse intracellular responses (Kenakin and Miller 2010). The challenge ahead will be to utilise our newly acquired knowledge of GPCR structures and of processes, such as biased agonism and allosterism, for the discovery and development of more selective and efficacious therapeutics.

Cardiovascular regulation and GPCRs

Cardiovascular homeostasis is regulated by an array of hormones and neurotransmitters, many of which exert their physiological actions via GPCRs expressed throughout the heart. Much of our knowledge relating to GPCRs and their signalling paradigms—from the early characterisation of ligand binding (Lefkowitz et al. 1974), receptor purification and cloning (Dixon et al. 1986; Murphy et al. 1991), identification of receptor polymorphisms that modulate signalling (Mason et al. 1999) through to pleiotropic/G protein-independent coupling (Lohse et al. 1990; Luttrell et al. 1999), biased signalling (Holloway et al. 2002; Thomas et al. 2000) and crystal structures (Cherezov et al. 2007; Rasmussen et al. 2011)—has stemmed from research on a small number of prototypical cardiovascular GPCRs, most prominently the β -adrenoceptor (β AR) and angiotensin II type 1 receptor (AT_1R)¹. These advances have been intimately tied to the importance of these

receptor systems in cardiac physiology and pathology [reviewed in Rockman et al. (2002); Salazar et al. (2007)]. Not surprisingly, several of the most successful cardiovascular therapeutics modulate adrenergic and angiotensin receptor signalling.

Adrenoceptors

Adrenoceptors are an important class of GPCRs and are responsible for translating chemical messages from the sympathetic nervous system into cardiovascular responses (Bylund et al. 1994). At least nine AR subtypes are expressed in mammalian hearts (3 α_1 ARs, 3 α_2 ARs and 3 β ARs) that mediate the effects of catecholamines (Perez et al. 2014). The α_1 ARs are expressed in the heart and vasculature and exert their vasoconstrictive effects via $G\alpha_q$ signalling pathways, whereas the α_2 ARs are primarily coupled to $G\alpha_i$ to reduce myocardial contractility and slow heart rate (Rockman et al. 2002). The β_1 AR is the predominant β AR subtype in the healthy heart and couples to $G\alpha_s$ -proteins to positively regulate inotropic and chronotropic responses, primarily via protein kinase A (PKA) phosphorylation of downstream effectors (Molenaar et al. 2000, 2007; Salazar et al. 2007). In the human heart, β_2 AR stimulation exerts maximal or near maximal effects on contractility through tight coupling to the stimulatory $G\alpha_s$ -protein–cyclic AMP–PKA pathway (Kaumann et al. 1999; Molenaar et al. 2000, 2007). The β_2 AR has also been reported to ‘switch’ its coupling to $G\alpha_i$ proteins to modulate receptor signalling and responses (Daaka et al. 1997) or simultaneously activate both $G\alpha_s$ and $G\alpha_i$ proteins (Kilts et al. 2000).

Alterations in β AR signalling are central to the pathogenesis of human heart failure where chronic elevation of catecholamines results in dysregulation of β AR signalling by receptor desensitisation and downregulation (Bristow et al. 1982, 1986), increased $G\alpha_i$ signalling (Neumann et al. 1988), and GRK upregulation (Bristow 2011; Rockman et al. 2002). The β AR has long been considered a prime target for the treatment of heart failure based on the discovery of propranolol as a β AR antagonist in 1964 (Black et al. 1964) and the pioneering use of the β -blockers practolol and alprenolol for the management of heart failure (Waagstein et al. 1975). Sympathetic nervous system activity is increased in human heart failure and is a negative prognostic indicator (Cohn et al. 1984). β -blockers work predominantly by inhibiting harmful β_1 AR-mediated effects of noradrenaline, leading to reductions in cardiac chronotropy and inotropy that reduce cardiac workload and metabolic demand (Violin et al. 2013). Nevertheless, although currently used β -blockers (e.g., carvedilol, metoprolol, bisoprolol) produce a survival benefit for heart failure patients (for review see Bristow 2000, 2011; Molenaar and Parsonage 2005), these compounds variously target the β_1 and β_2 ARs (Hoffmann et al. 2004; Molenaar

¹ A large proportion of this groundbreaking work was recognised with the awarding of the 2012 Nobel Prize for chemistry to Drs. R.J. Lefkowitz and B. Kobilka.

et al. 2006, 2013). For instance, although they are classified in the same class, carvedilol is approximately 13-fold selective for β_2 ARs (Molenaar et al. 2006), metoprolol is approximately 2.5-fold selective for β_1 ARs (Molenaar et al. 2013) and bisoprolol is approximately 50-fold selective for β_1 ARs (Hoffmann et al. 2004). These data provide compelling evidence that β -blockers exhibit biased signalling at the β ARs, which has important ramifications for the treatment of heart failure (Thanawala et al. 2014). Polymorphic variants in the β_1 AR also enhance β -blocker function in heart failure patients and thus could be exploited as a potential individualised treatment (Chen et al. 2007; Liggett et al. 2006; Mialet Perez et al. 2003).

As already mentioned, recent discoveries have elucidated many of the nuances of GPCR signal transduction, often focussing on the three β ARs. Indeed, an improved understanding of processes, ranging from the compartmentalisation and dynamic regulation of β AR signalling components (Fu et al. 2013; Molenaar et al. 2014; Nikolaev et al. 2010) to the diversity of G protein-dependent and independent signalling pathways (Lymperopoulos et al. 2012; Tilley 2011) and the possibility of biased signalling (DeWire and Violin 2011), may have future implications for the treatment of cardiovascular disease.

Angiotensin II type 1 receptor

There is a large and continually expanding literature on the renin–angiotensin system (RAS) and its contribution to broad aspects of cardiovascular, neural, endocrine and metabolic biology and disease (Bader 2010; de Gasparo et al. 2000; George et al. 2010; Oro et al. 2007). The principal bioactive peptide in the RAS, angiotensin II (AngII), is generated from its precursor angiotensinogen by renin and angiotensin-converting enzyme. AngII acts primarily at the AngII type I receptor (AT₁R) in vascular smooth muscle, kidney, brain and adrenal gland to influence vasoconstriction and sodium/water homeostasis, as well as promoting cardiac remodelling and hypertrophy. AT₁R is a $G_{\alpha_{q/11}}$ -coupled GPCR, whereby AngII stimulation leads to the activation of phospholipase C- β , hydrolysis of membrane phospholipids and the liberation of diacylglycerol and inositol trisphosphate. This results in activation of protein kinase C and intracellular calcium mobilisation, respectively (de Gasparo et al. 2000). AT₁R also couples to an array of other intracellular signal transduction pathways, including the RTK and mitogen-activated protein kinase (MAPK) pathways and those involved in the generation of reactive oxygen species and modulation of various ion channels (Oro et al. 2007). Resulting from decades of research on the RAS, pharmacological inhibitors of ACE and the non-peptide antagonists of the AT₁R (the ‘-sartans’) have become frontline therapeutics in the treatment of hypertension and CVD.

Moreover, some of the earliest work on biased agonism was conducted using a substituted form of angiotensin (Sar¹Ile⁴Ile⁸-AngII) that preferentially generates a signal via β -arrestin rather than via inositol phosphate after AT₁R activation (Holloway et al. 2002; Noda et al. 1996; Thomas et al. 2000). This work provided the lead compound for the development of a β -arrestin biased AT₁R ligand, TRV120027 (Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH) (Violin et al. 2010), which is currently in Phase 2 clinical trials for the treatment of acute heart failure (DeWire and Violin 2011). This compound effectively blocks the G protein-dependent AngII-mediated vasoconstriction, but increases cardiomyocyte contractility via β -arrestin signalling (Boerrigter et al. 2011, 2012; Monasky et al. 2013). Thus, by inhibiting the deleterious effects of increased AngII levels in the vasculature and kidney, while preserving cardiac output, this novel biased compound offers promise to abrogate the ‘on-target’ adverse effects of classical AT₁R ligands and therapeutics.

Muscarinic, adenosine, opioid and endothelin receptors

Beyond the adrenergic and angiotensin receptor systems, numerous other GPCRs have important functions in the heart. For example, resting heart rate is controlled by cholinergic signals mediated through muscarinic M₂ G_{α_i} -coupled receptors. These effects occur via inhibition of adenylyl cyclase and by $G\beta\gamma$ -inhibition of a potassium channel in the sinoatrial node (Logothetis et al. 1987; Rockman et al. 2002).

The four adenosine receptor sub-types (A₁, A_{2A}, A_{2B}, A₃) have been widely implicated in cardiovascular physiology, including the regulation of myocardial contraction, heart rate and conduction, coronary vascular tone, cardiac and vascular growth and cellular stress resistance (Headrick et al. 2013). A₁ and A₃ receptors signal primarily via G_{α_i} and are possible antiarrhythmic targets because they functionally oppose the effects of adrenoceptor signalling (Wilbur and Marchlinski 1997). Adenosine signalling and the A₁ and A₃ receptors are also important targets for cardioprotection in ischaemia/reperfusion injury (Donato and Gelpi 2003), with a recent study showing the benefit of an A₃-selective positive allosteric modulator in a canine infarction model (Du et al. 2012). Similarly, a novel pharmacological approach that combines allosteric modulation and biased agonism to target the A₁ receptor may hold therapeutic promise by promoting the beneficial cardioprotective effects without the confounding bradycardia mediated via the adenosine receptors (Valant et al. 2014).

Opioid receptors are expressed in the heart and can mediate paracrine effects on vascular tone, cardiac excitation–contraction coupling, heart rate and myocardial inotropy (Headrick et al. 2012; van den Brink et al. 2003). It is also interesting to note that the heart can synthesise and enzymatically process active opioid peptides, which in turn can act in an autocrine

manner (Headrick et al. 2012). Opioid receptors are also involved in cardioprotective responses to acute and chronic opioid treatment (Peart and Gross 2006).

Endothelin is a powerful vasoconstrictor that also has chronotropic, inotropic, direct arrhythmic, and hypertrophic effects in the cardiovascular system (Asano et al. 2002; Burrell et al. 2000; Kaoukis et al. 2013; Yamazaki et al. 1996). The activation of the endothelin-1 receptor (ET_A) promotes cardiac remodelling and hypertrophy via G α_q -mediated increases in intracellular Ca²⁺ and was proposed as a promising candidate for the treatment of heart failure (Spinale et al. 1997). Although subsequent clinical trials for ET_A antagonists were unsuccessful (Anand et al. 2004), endothelin signalling may still represent a viable target for resistant hypertension (Kaoukis et al. 2013).

Novel cardiovascular GPCR targets

Interestingly, while GPCRs are extremely successful drug candidates, current cardiovascular therapeutics target only a small subset of the known cardiac GPCRs. This deficiency has become more apparent in the post-genomic era, with the development of high-throughput technologies, such as microarrays and next-generation sequencing, that enable the profiling of GPCR expression in cells and tissues (Flegel et al. 2013; Min et al. 2010; Snead and Insel 2012; Spaethling et al. 2014). In an early global gene expression analysis of human tissues, the heart was reported to express upward of 150 different GPCRs (Hakak et al. 2003). Of these, only one-third had known ligands at the time (with the remainder designated ‘orphan receptors’), and yet a smaller subset of these was therapeutically targeted in the heart. These ‘new’ GPCRs were proposed as potential novel candidates for the modulation of heart function (Tang and Insel 2004), and the notion remains relevant today. Subsequent tissue profiling studies demonstrated that over 200 GPCRs are expressed in the adult mouse heart, with some differential expression between the right atria and the ventricles consistent with their specialised functions (Moore-Morris et al. 2009; Regard et al. 2008). Moreover, these analyses likely provide conservative estimates, as they excluded the odorant and taste receptor genes that account for around half of the GPCR superfamily (discussed below).

Most recently, transcriptomic analyses using RNA sequencing (RNA-seq) have suggested that many of the most highly abundant GPCRs in the heart have not necessarily attracted the attention of cardiovascular researchers (Flegel et al. 2013; highlighted in Table 1 and Fig. 2). The traditional focus on adrenergic and angiotensin receptors is due to their demonstrable contribution to cardiac function and the subsequent discovery/development of research tools to elucidate their roles. In contrast, many other GPCRs that are expressed in the heart require validation at the protein level, are orphans

and/or are experimentally intractable. Thus, the identification of selective ligands and the correlation of transcript-to-protein expression using quality antibodies and other robust assay systems are needed to define the cardiac role for these GPCRs. Importantly, these transcript data (Table 1, Fig. 2) come from one male (aged 77 years, of unknown cardiovascular health) and may therefore vary relative to the broader population. Nonetheless, it is reasonable to expect that the repertoire of expressed genes would be representative. While mammalian GPCRs (including the sensory receptors) share common ancestral genes, experimental studies in human cells and tissues are essential because of the relevance to human physiology, but also because separate rodent- and human-specific clades of these receptor genes exist with clear differences in their ligand binding properties (Foster et al. 2014b).

These GPCR expression profiling datasets reinforce the notion that there are many, as yet, unexplored potential receptor targets in the cardiovascular system. Recently, numerous additional GPCRs have been implicated in cardiovascular biology. For example, the protease activated receptor (PAR₁) has been variously associated with inflammation, pain and platelet activation and is therapeutically targeted to treat arterial thrombosis (Zhao et al. 2014). In addition, in an unbiased screen, PAR₁ was identified as the most highly expressed GPCR in adult rat cardiac fibroblasts, suggesting that it may be a potential candidate for the treatment of cardiac fibrosis (Snead and Insel 2012).

Likewise, relaxin was originally named for its vasodilatory role in the female reproductive tract, yet the members of relaxin family of peptide receptors (in particular RXFP1) have attracted increasing interest for their pleiotropic actions in the heart and cardiovascular system (Du et al. 2010). Relaxin has beneficial cardiac effects against inflammation, fibrosis, vasoconstriction and ischaemia, which have led to promising clinical trials for the treatment of acute heart failure (Teerlink et al. 2013).

The actions of apelin on its cognate receptor are also of interest in the cardiovascular context, particularly as apelin is one the most potent endogenous inotropic agents described to date, acting to increase contractility at sub-nanomolar concentrations (Maguire et al. 2009; Scimia et al. 2014; Szokodi et al. 2002). Interestingly, the apelin receptor is also activated by mechanical stretch in a ligand-independent manner, resulting in a receptor conformation favourable for β -arrestin recruitment and signalling (Scimia et al. 2012). From a therapeutic standpoint, these findings are important as stretch activation of the apelin receptor in the heart can be deleterious. It would therefore be desirable to selectively inhibit the stretch activation or bias towards the apelin and G protein-mediated signalling responses.

A number of orphan GPCRs have also been implicated in cardiovascular control—including those in development, for example GPR161 (Leung et al. 2008), and as novel targets in heart failure. Two such candidates, GPR37L1 (downregulated

Table 1 Transcripts of the most abundant G protein-coupled receptors in human heart tissue taken from a 77-year-old man

Receptor gene name ^a	FPKM ^b	GPCR symbol ^c	Family	Endogenous ligand(s)
Neuropeptide Y receptor type 6	93.02	Y ₆ receptor	Rhodopsin (Class A)	None ^d
Sphingosine-1-phosphate receptor 1	42.52	S1P ₁ receptor	Rhodopsin	Sphingosine 1-phosphate
G protein-coupled receptor 116	39.50	GPR116	Adhesion	Orphan
G protein-coupled receptor, family C, group 5, member B	34.74	GPRC5B	Glutamate (Class C)	Orphan
Endothelin receptor type B	18.52	ET _B receptor	Rhodopsin	Endothelin
G protein-coupled receptor 133	18.35	GPR133	Adhesion	Orphan
Frizzled family receptor 4	18.27	FZD ₄	Frizzled	Wnt
Cholinergic receptor, muscarinic 2	17.31	M ₂ receptor	Rhodopsin	Acetylcholine
Sphingosine-1-phosphate receptor 3	17.22	S1P ₃ receptor	Rhodopsin	Sphingosine 1-phosphate
G protein-coupled receptor 137	16.32	GPR137	Other	Orphan
Latrophilin 2	16.04	LPHN2	Adhesion	Orphan
G protein-coupled receptor 56	15.71	GPR56	Adhesion	Orphan
CD97 molecule	15.07	CD97	Adhesion	Orphan
Adrenoceptor beta 1	14.84	β ₁ -adrenoceptor	Rhodopsin	(-)-adrenaline, (-)-noradrenaline
G protein-coupled receptor 137B	14.56	GPR137B	Other	Orphan
Atypical chemokine receptor 3	13.80	ACKR3	Rhodopsin	CXCL11, SDF-1α
Formyl peptide receptor 1	13.71	FPR1	Rhodopsin	Annexin-1, cathepsin G
G protein-coupled receptor 108	13.10	GPR108	Rhodopsin	Orphan
Frizzled family receptor 1	12.58	FZD ₁	Frizzled	Wnt
Leucine-rich repeat containing G protein-coupled receptor 4	12.19	LGR4	Rhodopsin	R-spondins
G protein-coupled receptor 107	12.03	GPR107	Rhodopsin	Neuronostatin
Histamine receptor H2	11.20	H ₂ receptor	Rhodopsin	Histamine
Calcitonin receptor-like receptor	10.87	CALCRL	Secretin (Class B)	adrenomedullin, CGRP
Coagulation factor II (thrombin) receptor	10.18	PAR1	Rhodopsin	Thrombin
G protein-coupled receptor 17	9.16	GPR17	Rhodopsin	Cysteinyl-leukotrienes (CysLTs), uracil nucleotides
Adrenoceptor alpha 1A	8.82	α _{1A} -adrenoceptor	Rhodopsin	(-)-adrenaline, (-)-noradrenaline
G protein-coupled receptor 22	8.70	GPR22	Rhodopsin	Orphan
G protein-coupled receptor 124	8.64	GPR124	Adhesion	Orphan
G protein-coupled receptor 146	8.58	GPR146	Rhodopsin	Orphan
G protein-coupled receptor 125	7.86	GPR125	Adhesion	Orphan

Data have been curated from the publicly available Illumina Human BodyMap 2.0 project RNA-seq dataset (adapted from Flegel et al. 2013)

^a Gene names are from the HUGO Gene Nomenclature Committee (<http://www.genenames.org/>)

^b Gene expression for each tissue is presented based on the number of fragments per kilobase of exon per million fragments mapped (FPKM)

^c GPCR (G protein-coupled receptor) symbols are from <http://www.iuphar-db.org/>

^d Pseudogene in humans

in CVD) and GPR35 (upregulated in myocardial infarction), were identified by correlating gene expression level with clinical parameters, such as pulmonary artery pressure, left ventricular ejection fraction and brain natriuretic peptide mRNA level (Min et al. 2010). Indeed, GPR35 may be a potential prognostic marker for cardiac failure (Ronkainen et al. 2014), but resolution of its function is confounded by a complex pharmacology with regard to potential endogenous and synthetic ligands, as well as marked species differences in G protein-dependent and -independent signalling assays (Jenkins et al. 2012; Neetoo-Isseljee et al. 2013).

Odorant and taste receptors in the heart

Since the discovery of large multigene GPCR families as the molecular mediators of olfaction and taste (Adler et al. 2000; Buck and Axel 1991; Nelson et al. 2001, 2002), odorant and taste receptors have also been identified in diverse physiological contexts. Accumulating evidence suggests that these receptors perform additional biological roles outside the nose and mouth (Foster et al. 2014b). In the course of our work investigating cellular growth pathways in the heart (Chan et al. 2006; Smith et al. 2011; Thomas et al. 2002), we performed microarrays to examine and compare the hypertrophic gene

Fig. 2 All GPCR families are represented in the heart. **a** Schematic showing the proportion of GPCRs classified into each receptor family. **b** Number of GPCRs from each receptor family expressed in human heart tissue taken from a 77-year-old man. Odorant receptors (*ORs*) are the

programs activated by AngII and epidermal growth factor ligands. Surprisingly, we found that odorant receptor transcripts (as well as the olfactory G protein, $G\alpha_{olf}$ and adenylyl cyclase 3) were expressed in isolated and purified rat cardiomyocytes, and some were regulated in response to hypertrophic stimuli (unpublished results). This is consistent with other published microarray datasets (Zhang et al. 2004, 2007) that show there are numerous odorant receptors expressed in the cardiovascular system and indeed some that appear exclusively expressed in the heart. Similarly, two independent studies identified transcripts for a single odorant receptor gene (*Olr1654*) in rat heart, although no functional experiments were pursued (Drutel et al. 1995; Ferrand et al. 1999). These findings portend interesting and novel underlying cardiac biology, a notion reinforced by the attribution of new roles for odorant receptors in skeletal muscle, prostate cancer and kidney (Griffin et al. 2009; Neuhaus et al. 2009; Pluznick et al. 2009, 2013).

We have also recently provided the first description of taste receptors in rodent and human hearts (Foster et al. 2013). Using a combination of approaches, we demonstrated the expression of individual taste receptors (i.e. members of both the TAS1R and TAS2R family of GPCRs) in cardiac cells and tissues. These receptors were expressed in a small subset of cells throughout the myocardium, with some receptors upregulated at the transcript level in vitro and in vivo upon nutrient deprivation (Foster et al. 2013). TAS2R transcripts were readily detected in human heart tissue samples obtained from heart transplant patients, notably at comparable abundance to the classically targeted angiotensin (AT_1) and β_1 -adrenergic receptors (Foster et al. 2013). The apparent responsiveness of the taste receptors to nutrient status in the heart has compelled us to investigate their regulation in CVD. In collaboration with the Sydney Heart Bank, we have profiled the expression of taste receptors in healthy and diseased hearts from patients of various aetiologies and ages. To date, we have not observed any obvious taste receptor gene regulation with disease,

although the analyses are ongoing. Irrespective of changes in transcript abundance in heart disease, the most important regulation of GPCR function occurs at the protein level, generally in relation to receptor localisation and ligand availability. We are currently interested in the potential function of taste receptors in human cardiac contractility, as we have recently shown in rodents (Foster et al. 2014a). Accordingly, studies are underway aimed at investigating the contractile responses to selective TAS2R ligands in patient-derived human right atrial tissue.

Coincidentally, the study of extraoral taste receptors has captured the attention of the broader research community. Several important studies have now appeared in the literature detailing the function of specific taste receptors in the gastrointestinal tract (Jang et al. 2007; Mace et al. 2009), airway smooth muscle (Civelli et al. 2013; Deshpande et al. 2010) and upper respiratory tract (Lee et al. 2012; Tizzano et al. 2010), with roles in regulating gut hormone release and nutrient absorption, bronchodilation and innate defence, respectively. In the latter case, a specific taste receptor (TAS2R38) is activated by quorum-sensing molecules secreted from bacteria, contributing to a nitric oxide-mediated endogenous defence response. In addition, patients who possess a non-functional polymorphic receptor variant have an increased susceptibility to respiratory infection (Lee et al. 2012). Thus, the ascription of novel functions to these receptors, with direct pathophysiological relevance, provides compelling justification for the further characterisation of taste receptors in the heart.

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Closing remarks and perspectives

There is little doubt that GPCRs have proven to be very successful drug targets for the modulation of cardiovascular function. At present, the angiotensin and adrenergic receptor

systems are well-established therapeutic targets in the management of hypertension, coronary artery disease and treatment of heart failure. Nonetheless, as the incidence of CVD continues to rise, the prognosis for heart failure remains poor and, therefore, it is clear that there is room for improvement. To this end, there remains great interest in translating GPCR research into clinical trials by employing new approaches, such as biased ligands at ‘classical’ targets (e.g. TRV120027 for the AT₁R), and by developing new candidates, such as the relaxin/RXFP1 system. In addition, we now realise that there are many receptors that have yet to be fully characterised in the heart, especially orphan, odorant and taste GPCRs. Indeed, where the endogenous activating ligands are unknown, it may be better to regard odorant and taste receptors as *orphan* GPCRs. Finally, by gaining an improved understanding of the signalling of these receptors in the heart, it is likely that additional/complementary GPCR targets to modulate cardiac physiology and pathophysiology will be identified.

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Compliance with Ethical Standards

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Conflict of interest Thomas G. Walter, Simon R. Foster, and Roura Eugeni declare that they have no conflict of interest.

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