UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Regulation of cardiovascular GPCR signalling

Balk, M.C.

Publication date
2009

Link to publication

Citation for published version (APA):
Balk, M. C. (2009). *Regulation of cardiovascular GPCR signalling*. [Thesis, fully internal, Universiteit van Amsterdam].

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:09 Mar 2023

1

Regulation of G protein-coupled receptor signalling: Focus on the cardiovascular system and regulator of G protein signalling proteins. A general introduction

Mariëlle C. Hendriks-Balk, Stephan L. Peters, Martin C. Michel, Astrid E. Alewijnse

European Journal of Pharmacology 2008; 585(2-3):278-291

ABSTRACT

G protein-coupled receptors (GPCRs) are involved in many biological processes. Therefore, GPCR function is tightly controlled both at receptor level and at the level of signalling components. Well known mechanisms by which GPCR function can be regulated comprise desensitization/ resensitization processes and GPCR up- and down-regulation. GPCR function can also be regulated by several proteins that directly interact with the receptor and thereby modulate receptor activity. An additional mechanism by which receptor signalling is regulated involves an emerging class of proteins, the so-called regulators of G protein signalling (RGS). In this review we will describe some of these control mechanisms in more detail with some specific examples in the cardiovascular system. In addition, we will provide an overview on RGS proteins and the involvement of RGS proteins in cardiovascular function.

INTRODUCTION

G protein-coupled receptors (GPCRs) are the largest cell surface receptor superfamily and also the largest class of drug targets to which ligands are currently directed. GPCRs, of which more than 800 are known in the human genome, are involved in virtually every physiological process (Fredriksson et al., 2003). In the cardiovascular system GPCRs play a pivotal role by regulating essential cardiovascular functions such as heart rate and contractility and vascular tone. More than 100 different GPCRs are expressed in the cardiovascular system (Tang and Insel, 2004). Among them are the adrenoceptors, angiotensin II receptors, endothelin receptors, muscarinic acetylcholine receptors, serotonin receptors, bradykinin receptors, adenosine receptors, vasopressin receptors, lysophosphatidic acid receptors and sphingosine-1-phosphate receptors (Tang and Insel, 2004).

As GPCRs are essential in many biological processes, GPCR desensitization, including internalization, GPCR expression and responsiveness are tightly controlled (see Fig. 1). Regulation at the GPCR level comprises desensitization and resensitization processes which change the availability of receptors to be stimulated, and up- or down-regulation of receptor expression which changes the amount of GPCRs. Control of GPCR responsiveness involves the regulation of signalling proteins, e.g. G proteins, protein kinases, phospholipases.

In this review we will first describe GPCR signalling and GPCR desensitization in general with some specific examples in the cardiovascular system. Also the involvement of changes in GPCR desensitization, expression of GPCRs and of some signalling components in cardiovascular diseases will be described. The second part will focus on GPCR interacting proteins, especially the regulator of G protein signalling (RGS) proteins, and the impact of these RGS proteins on GPCR signalling in the cardiovascular system and in cardiovascular diseases.

GPCR SIGNALLING

GPCRs are membrane-spanning proteins with seven transmembrane domains that respond to various stimuli such as light, tastes, odors, hormones, neurotransmitters, of various chemical classes including calcium ions, peptides, amino acids, nucleotides, lipids and fatty acid derivatives (for extensive review see (Kristiansen, 2004)). The seven α-helical transmembrane domains are connected by three intracellular and three extracellular loops. Based on the primary sequence, GPCRs can be divided in at least three subfamilies. The rhodopsin-like family A is the largest and best-studied subfamily and includes the aminergic receptors, peptide receptors, rhodopsin and olfactory receptors. The other two main subfamilies are the secretin-like receptor family B (which bind several neuropeptides and other peptide hormones) and the metabotropic glutamate receptor-like family C.

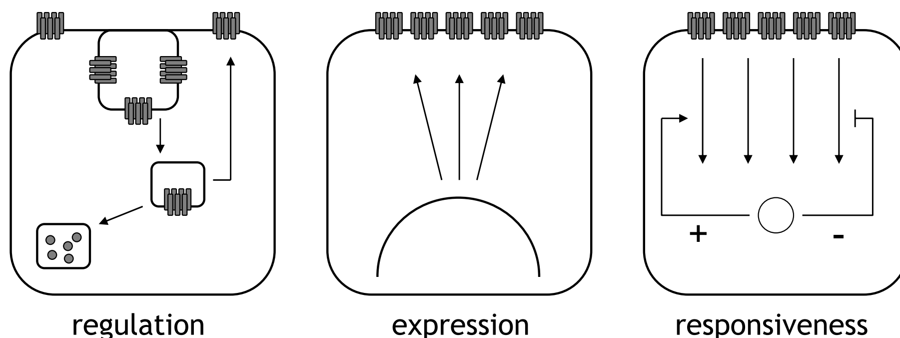

Fig. 1. GPCR signalling is tightly controlled. Regulation at the receptor level comprises GPCR desensitization, including internalization, and GPCR expression, which can be up- or down-regulated. Regulation at the level of signalling proteins, e.g. by specific protein interactions with the receptor, can change GPCR responsiveness.

The proposed general receptor structure of transmembrane domains with extra- and intracellular loops was confirmed by the crystallization of GPCRs. To date, only two GPCRs have been crystallized. In 2000 the three dimensional crystal structure of bovine rhodopsin was obtained (Palczewski et al., 2000). Very recently, two studies reported on the crystallization of the β_2-adrenoceptor (Cherezov et al., 2007; Rasmussen et al., 2007).

G proteins

GPCRs transfer extracellular signals across the plasma membrane to intracellular effectors via G proteins. The binding site for small ligands such as the biogenic amines, is mainly located at a pocket within the helical bundle whereas larger ligands, like peptides and large proteins, interact with the extracellular parts of the receptor, especially with the N-terminal tail, which is generally larger for these receptors (Bockaert and Pin, 1999; Gether, 2000; Kristiansen, 2004). Agonist binding induces a conformational change of the GPCR allowing heterotrimeric G proteins to interact with the GPCR (for extensive review on GPCR signalling see (Cabrera-Vera et al., 2003; Kristiansen, 2004; Pierce et al., 2002)). Most importantly, the second and third intracellular loop and the C-terminal tail of the receptors are important for the interaction with G proteins (Gether, 2000; Wess, 1997; 1998). In the last two decades it has become clear that many GPCRs not only transmit signals after stimulation by agonists but can also spontaneously couple to signal-transduction pathways, i.e. many GPCRs can be present in a constitutive or spontaneously active conformation that interacts with and activates G proteins (Milligan, 2003; Smit et al., 2007).

G proteins consist of a GDP/GTP-binding α-subunit and a $\beta\gamma$-subunit complex. In mammalian cells 16 genes encode for Gα-subunits, at least 5 genes for Gβ-subunits and 12 genes for Gγ-subunits (Cabrera-Vera et al., 2003; Milligan and Kostenis, 2006). The heterotrimeric G

proteins are divided into four families: G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$ based on similarity of α-subunits (Hamm, 1998).

GPCRs show preferences for a specific G protein, although no consensus sequences for G protein selectivity have been clearly identified (Moller et al., 2001; Wess, 1998). However, several GPCRs have been identified to signal through more than one Gα subunit (Hermans, 2003), such as the M_2 muscarinic receptor which can couple to G_i, G_s and $G_{q/11}$ (Michal et al., 2007) or the sphingosine-1-phosphate receptors $S1P_2$ and $S1P_3$ which couple to $G_{q/11}$, G_i and $G_{12/13}$ (Siehler and Manning, 2002). In addition, different agonists that bind to the same receptor are able to stimulate distinct G protein signalling pathways, a phenomenon called agonist-trafficking, ligand-directed signalling or functional selectivity (Kenakin, 2001; 2007; Maudsley et al., 2005; Michel and Alewijnse, 2007; Urban et al., 2007). Similarly, signal-selective antagonism, in which an antagonist blocks only a subset of agonist-induced signalling pathways, has been described for some GPCRs (Baker and Hill, 2007; Pommier et al., 1999). Overall these findings can be explained by the existence of multiple active conformations of the receptor, which might be ligand-specific (Ghanouni et al., 2001; Kobilka and Deupi, 2007). The functional selectivity of GPCRs differs among different cell types expressing the same receptor (Kenakin, 2003). This might be due to differences in expression and/or compartmentalization of the signalling components in the different cell types (Kenakin, 2002). However, differences in receptor densities are also known to affect functional selectivity (Michel and Alewijnse, 2007; Sato et al., 2007).

Interaction with an agonist-activated GPCR triggers the GDP-GTP exchange on the α-subunit, thereby generating active GTP-bound Gα subunit from the inactive GDP-bound state followed by dissociation from the receptor and Gβγ subunit. Both the Gα and Gβγ subunit can modulate the activity of downstream effectors. Specific Gα subunits generate different responses (for extensive review see (Cabrera-Vera et al., 2003). For instance when coupled to G_s or $G_{i/o}$ proteins GPCRs either activate or inhibit adenylyl cyclase (AC) activity resulting in an increase or decrease in cAMP formation, respectively (Cabrera-Vera et al., 2003). Cyclic AMP can subsequently activate cAMP-dependent protein kinase (PKA) and the Rap guanine exchange factor Epac (exchange protein activated by cAMP) (Schmidt et al., 2007). In addition, $G_{i/o}$ proteins are involved in the inhibition of Ca^{2+} channels, the stimulation of G protein inwardly rectifying K^+ (GIRK) channels and the stimulation of phosphodiesterases (Pierce et al., 2002). Interaction with $G_{q/11}$ stimulates phospholipase C (PLC) leading to the formation of inositol triphosphate (IP_3) and diacylglycerol followed by an increase in intracellular calcium ions and activation of protein kinase C (PKC), respectively. $G_{12/13}$ activation mainly leads to activation of small G proteins like RhoA via Rho guanine nucleotide exchange factors (RhoGEF) (Cabrera-Vera et al., 2003). Dissociated Gβγ subunits also target a range of signalling pathways involved in proliferation, apoptosis and ion channel activation, such as mitogen-activated protein kinases (MAPKs), phosphatidylinositol-3-kinase (PI3K), PLCβ, AC and the small GTP binding proteins (Cabrera-Vera et al., 2003; Schwindinger and Robishaw,

2001). In addition, dissociated Gβγ subunits recruit proteins involved in GPCR desensitization and down-regulation (Desai et al., 2006).

The rate and duration of G protein signalling is determined by the lifetime of the active GTP-bound Gα subunit. GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the Gα subunit, resulting in re-association of the Gα subunit with the Gβγ subunit and termination of the G protein signalling. The intrinsic GTPase activity of Gα subunits can be accelerated by the presence of GTPase-activating proteins (GAPs), such as regulator of G protein signalling (RGS) proteins, which are described below. Also, several second messenger proteins, such as some PLC isoforms, show GAP activity towards the G protein (Chidiac and Ross, 1999).

GPCR signalling in the cardiovascular system

As already mentioned before GPCRs regulate essential functions in the cardiovascular system, such as heart rate and contractility and vascular tone. Contraction in the vascular system is mostly mediated by $G_{q/11}$ signalling of GPCRs, PLC activation and subsequent IP_3 and diacylglycerol formation (Allen and Walsh, 1994). Activation of IP_3 receptors results in a release of calcium into the cytosol. Increased calcium levels then promote the formation of calcium-calmodulin complexes, which in turn activate myosin light chain kinase to phosphorylate myosin light chains, causing contraction of vascular smooth muscle cells. In addition, PKC activation by diacylglycerol may lead to inhibition of myosin phosphatase resulting in a decreased dephosphorylation of myosin light chains, thereby enhancing the contraction of the vascular smooth muscle cells. Also $G_{i/o}$-mediated activation of PLC and inhibition of AC or $G_{12/13}$–mediated Rho and Rho kinase activation in vascular smooth muscle cells may lead to an increase in vascular tone (Maguire and Davenport, 2005). Several GPCRs, such as the angiotensin II AT_1, endothelin-1 ET_A receptors and α_1-adrenoceptors mainly use the $G_{q/11}$-PLC signalling pathway to stimulate vasoconstriction whereas other receptors, such as the α_2-adrenoceptors, mediate contraction via $G_{i/o}$ proteins (Maguire and Davenport, 2005).

Relaxation of vascular smooth muscle cells is mainly mediated by G_s protein-coupled receptors, which reduce the Ca^{2+} sensitivity. In addition, $G_{q/11}$ and $G_{i/o}$ protein signalling in the endothelial cells also stimulate relaxation in the vascular smooth muscle cells via the release of nitric oxide, prostaglandins and endothelium-derived hyperpolarizing factors (EDHFs). Nitric oxide acts directly on guanylyl cyclase, resulting in increased cGMP levels in the vascular smooth muscle cell. Prostaglandins reduce Ca^{2+} sensitivity via G_s protein-coupled receptors on the vascular smooth muscle cell, whereas EDHFs act on ion channels to cause hyperpolarization in the vascular smooth muscle cell (Maguire and Davenport, 2005). All these events lead to relaxation of the vascular smooth muscle cell. Well-known GPCRs mediating vasodilation are the β-adrenoceptors on the vascular smooth muscle cells and the muscarinic M_1 and M_3 receptors on the endothelial cells. Intriguingly, some receptors such as

angiotensin II receptors can promote both vasoconstriction and vasodilatation by acting on vascular smooth muscle and endothelial cells, respectively (Mulders et al., 2006).

In cardiomyocytes contraction is mainly mediated by G_s signalling, involving PKA phosphorylation of several proteins including several Ca^{2+} channels. This phosphorylation leads to an increase in intracellular calcium levels resulting in increased contraction (positive inotropic effect) (Zaugg and Schaub, 2004). $G_{i/o}$ signalling decreases PKA activity resulting in inhibition of calcium effects and thus reduced contractile force (negative inotropic effect). Cardiomyocyte contraction is predominantly mediated via the β_1-adrenoceptor by sympathetic activity and to a lesser extent via the β_2-adrenoceptor (Brodde et al., 2006) whereas the reduced contractile force is mainly mediated via the muscarinic M_2 receptor by parasympathetic activity (Brodde and Michel, 1999). In addition, increased cAMP levels in cardiac pacemaker cells causes activation of hyperpolarization-activated cyclic nucleotide-regulated cation channels (HCN channels) resulting in depolarization of the cells and thus an increase in heart rate (positive chronotropic effect) (Zaugg and Schaub, 2004).

A decrease in heart rate (negative chronotropic effect) is caused by $G_{i/o}$-mediated inhibition of AC and inhibition of HCN channel activity, and by $G\beta\gamma$-mediated activation of GIRK channels and subsequent hyperpolarization in pacemaker cells (Zaugg and Schaub, 2004). The heart rate is thought to be controlled predominantly by the β_2-adrenoceptor (positive chronotropic) and the muscarinic M_2 receptor (negative chronotropic) (Brodde et al., 2006; Brodde and Michel, 1999). $G_{q/11}$-coupled GPCRs, such as the angiotensin AT_1 and the endothelin ET_A receptors, are also able to induce positive inotropic and chronotropic effects in cardiomyocytes; the $G_{q/11}$-coupled α_1-adrenoceptors can cause positive inotropic effects in several species but play only a marginal role in the human heart (Brodde and Michel, 1999). Other GPCRs expressed in cardiomyocytes are suggested to influence the signalling of the adrenoceptors and vice versa in several ways, such as counteracting the signalling, stimulating dephosphorylation of signalling proteins, receptor phosphorylation and internalization (Dzimiri, 2002).

REGULATION OF GPCRS

Receptor desensitization by phosphorylation

Agonist binding to GPCRs triggers not only their activation but also cellular events leading to rapid attenuation of receptor responsiveness, called desensitization (for extensive review see (Ferguson, 2001; Marchese et al., 2008; Premont and Gainetdinov, 2007)). The first step in desensitization is phosphorylation of the receptor by second messenger-dependent protein kinases, such as PKA and PKC, or by GPCR kinases (GRKs), resulting in uncoupling of the receptor from the G protein. The targets for phosphorylation are the serine and/or threonine

residues in the third intracellular loop and/or the C-terminal tail of the receptor. GRKs phosphorylate the GPCR at serine and/or threonine residues different from those phosphorylated by second messenger-dependent protein kinases (Moffett et al., 1993; Mundell et al., 2003).

Since stimulation of GPCRs may lead to PKA or PKC activation via AC and PLC, respectively, phosphorylation by PKA or PKC provides a negative feedback mechanism of receptor stimulation (Benovic et al., 1985; Tobin, 1997). However, PKA and PKC are also able to phosphorylate other unstimulated GPCRs, thus causing desensitization of a GPCR as a result of stimulation of another GPCR (heterologous desensitization) (Lee and Fraser, 1993; Miyoshi et al., 2004; Mundell et al., 2004). It is suggested that phosphorylation of activated GPCRs by second messenger-dependent protein kinases is mainly important at low agonist concentrations whereas at higher agonist concentrations receptor phosphorylation is dominated by GRK activity (Balmforth et al., 1997; Tobin, 1997). In cardiomyocytes PKA-mediated phosphorylation of the β_2-adrenoceptor is involved in the attenuation of its inotropic effects and causes a shift in G protein-coupling selectivity towards G_i proteins (Daaka et al., 1997a). This shift from G_s towards G_i protein signalling further attenuates the positive inotropic effects of the β_2-adrenoceptor.

In contrast to phosphorylation by second messenger-dependent protein kinases, phosphorylation of GPCRs by GRKs only occurs at agonist-bound receptors. Upon agonist binding GRKs are recruited to the activated receptor, which in turn is phosphorylated resulting in uncoupling of the G protein. Phosphorylation by GRK promotes the binding of arrestins to the receptor (Moore et al., 2007).

The GRK family consists of seven GRK proteins of which GRK2, 3, 5 and 6 are ubiquitously expressed (Penela et al., 2006; Ribas et al., 2007). GRK2 and 3 are cytosolic proteins that are recruited to the cell membrane in part by the dissociated $G\beta\gamma$ subunits upon receptor stimulation (Daaka et al., 1997b). GRK5 and 6 are constitutively localized at the plasma membrane, either by electrostatic interaction or palmitoylation respectively (Pitcher et al., 1996; Stoffel et al., 1994). The GRKs show some selectivity towards specific GPCRs (Pitcher et al., 1998), although the structural features of the activated GPCR that are recognized by the GRKs are still unknown.

In the cardiovascular system GRK2 is important for the regulation of several receptor responses and GRK2 deletion is lethal due to hypoplasia of the ventricular myocardium and subsequent heart failure (Jaber et al., 1996). Reduced levels of GRK2, as in heterozygous GRK2 knockout mice, lead to an increase in β-adrenoceptor-mediated positive inotropic effects (Iaccarino and Koch, 2003). Also GRK3 and GRK5 are expressed in the cardiovascular system and have been shown to regulate some receptor responses. GRK3 shows selectivity towards thrombin receptors and α_{1B}-adrenoceptors, whereas GRK5 shows selectivity towards angiotensin II receptors and β-adrenoceptors as demonstrated in cardiac-specific transgenic mice (Iaccarino and Koch, 2003). Overall, GRK2 seems to be essential in the regulation of several cardiovascular responses, and GRK3 and GRK5 are involved in the desensitization of some specific GPCRs.

Internalization

Agonist stimulation for a period of minutes to several hours results in internalization of the GPCR, which is a well-known desensitization mechanism for many GPCRs (for extensive reviews see (Ferguson, 2001; Marchese et al., 2008)). Several different pathways of internalization have been described, including clathrin-coated pits and lipid rafts or caveolae (Kirkham and Parton, 2005).

The best characterized mechanism of GPCR internalization is via clathrin-coated pits. Phosphorylation by GRK facilitates the high affinity binding of β-arrestin1 or β-arrestin2 (also named arrestin2 and 3) to the receptor, preventing further G protein coupling (Moore et al., 2007). In contrast, phosphorylation of the receptor by second messenger-dependent protein kinases does not promote β-arrestin binding (Moore et al., 2007). β-Arrestins physically link GPCRs to proteins involved in receptor trafficking, such as the adaptor protein AP2, clathrin and dynamin (Shenoy and Lefkowitz, 2003a). These interactions are critical for recruitment of the GPCR into clathrin-coated pits at the cell surface as well as for receptor internalization.

Interestingly, not all receptors need to be phosphorylated or bind to arrestins to internalize. For example the $S1P_3$ receptor has been shown to be internalized without phosphorylation (Rutherford et al., 2005). The metabotropic glutamate receptor 1A (Dale et al., 2001) and the protease-activated receptor 1 (Paing et al., 2002) can be internalized without arrestin binding.

Thus, GPCRs can also be internalized via mechanisms that do not include β-arrestin binding and clathrin-coated pits. An example of another mechanism is the internalization via lipid rafts and/or caveolae. Although the precise mechanism of internalization via lipid rafts and/or caveolae is still unknown, it is thought to involve GPCR phophorylation by second messenger-dependent protein kinases and targeting to caveolae (Rapacciuolo et al., 2003) where many signalling components are localized (Insel et al., 2005b). Agents that disrupt lipid rafts and caveolae have been shown to prevent the internalization of several GPCRs, including the endothelin ET_B receptor and some vasoactive intestinal peptide (VIP) receptors (Claing et al., 2000).

After internalization, the GPCR can either be dephosphorylated and recycled back to the cell surface or targeted to lysosomes for degradation. β-Arrestins have been shown to differentially regulate GPCR trafficking after internalization. Some GPCRs recycle rapidly to the cell surface after internalization, such as the β_2-adrenoceptor, whereas other receptors recycle more slowly, such as the angiotensin II AT_{1A} and vasopressin V_2 receptor (Shenoy and Lefkowitz, 2003b). This difference in recycling rate is caused by the difference in stability of the GPCR-arrestin complex. The β_2-adrenoceptor appears to form a relatively unstable complex with β-arrestin (in most cases β-arrestin2) that dissociates near the plasma membrane and subsequently results in rapid recycling of the receptor (Shenoy and Lefkowitz, 2003b). However, angiotensin II AT_{1A} and vasopressin V_2 receptors form relative stable complexes with either β-arrestin1 or β-arrestin2 that internalize together into endocytic vesicles and remain

associated for longer times (up to 4 h) before recycling to the cell surface (Martin et al., 2003; Shenoy and Lefkowitz, 2003b). Upon binding to the GPCR β-arrestin is ubiquitinated which is transient in the unstable β-arrestin-GPCR complex and prolonged in the stable complex (Shenoy and Lefkowitz, 2003b). Ubiquitination and dephosphorylation of β-arrestin, which is constitutively phosphorylated in the cytosol (Lin et al., 1997), is required for the internalization of the receptor (Lin et al., 1997; Shenoy and Lefkowitz, 2003b). The GPCR can also be ubiquitinated, but this ubiquitination is not required for receptor internalization. Ubiquitination of the GPCR, however, regulates the lysosomal sorting and subsequent degradation of the activated GPCR (Marchese et al., 2008; Martin et al., 2003; Shenoy and Lefkowitz, 2005; Shenoy et al., 2001).

In the cardiovascular system β-arrestin1 is involved in the desensitization of positive inotropic effects of β-adrenoceptors as shown by increased β-adrenoceptor-mediated effects in β-arrestin1 knockout mice. However, chronotropic effects mediated via β-adrenoceptors were not affected in these knockout mice (Conner et al., 1997). β-Arrestin2, together with GRK6, was shown to be involved in the $G_{q/11}$-independent AT_{1A} receptor-mediated inotropic effects in cardiomyocytes (Rajagopal et al., 2006).

Receptor down-regulation

Almost all known GPCRs show some degree of down-regulation upon chronic agonist treatment (Bouvier et al., 1989; Yoburn et al., 2004). Down-regulation of receptor protein expression may not only be due to enhanced GPCR internalization and subsequent degradation, but also to decreased levels of receptor mRNA, either as a result of suppressed gene expression or destabilization of receptor mRNA (Hadcock and Malbon, 1988; Hadcock et al., 1989). In some cases chronic treatment with antagonists or inverse agonists will result in an up-regulation of GPCRs. This up-regulation is most likely due to enhanced protein synthesis or an increase in GPCR trafficking to the cell surface (MacEwan and Milligan, 1996; Yoburn et al., 2004). Like GPCR signalling, GPCR desensitization mechanisms might be ligand- and cell type-specific (Kelly et al., 2008; Menard et al., 1997).

ALTERED EXPRESSION LEVELS IN CARDIOVASCULAR DISEASES

Signalling by GPCRs is highly regulated by a range of physiological and pathophysiological conditions. One critical way in which a cell controls its response to extracellular GPCR ligands is through regulation of the expression and activity of each component of the GPCR-G protein-effector pathway.

Altered GPCR expression

Several disease states are known to be associated with altered GPCR expression. Up-regulation of the AT_1 receptor was demonstrated in atherosclerotic tissue with high correlation between AT_1 receptor expression and the severity of atherosclerosis (Gross et al., 2002). In congestive heart failure a down-regulation of the β_1-adrenoceptor (Brodde, 1991), the muscarinic M_2 receptor (Le Guludec et al., 1997) and the angiotensin II AT_2 receptor (Kacimi and Gerdes, 2003) expression was shown, reducing functional responsiveness of these receptors. In contrast, the β_3-adrenoceptor density is increased in heart failure (Moniotte et al., 2001) and the AT_1 receptor is up-regulated in left ventricular hypertrophy but returns to normal expression levels in the progression to congestive heart failure (Kacimi and Gerdes, 2003). There are indications that mRNA levels of several other GPCRs, such as the muscarinic M_3 and M_4 receptors and the adrenoceptors, are altered in congestive heart failure (Brattelid et al., 2007). However, there are some pitfalls when measuring regulation of mRNA expression, e.g. the normalization factor (Hendriks-Balk et al., 2007). In addition, changes in mRNA expression do not necessarily result into changes of the corresponding protein. Hence many reports on altered mRNA expression levels have to be interpreted with caution

Altered expression of GPCR signalling and desensitization components

In addition, protein expression of cardiac G_i (El-Armouche et al., 2003; Schnabel and Bohm, 1996) and G_{13} are increased in congestive heart failure whereas G_q and G_{12} are decreased (Kacimi and Gerdes, 2003). Other changes in congestive heart failure and cardiac hypertrophy include up-regulation of GRK2, associated with attenuation of β_1- and β_2-adrenoceptor signalling and reduction in cardiac output (Metaye et al., 2005; Penela et al., 2006). In some animal models of heart failure also an increase in GRK5 expression and activity was found, although its role in human heart failure remains unclear (Vinge et al., 2001). In addition, both GRK2 and GRK5 up-regulation has been associated with hypertension in animal studies, which might be due to increased desensitization of receptors for vasodilators, e.g. the β-adrenoceptors (Gros et al., 2000; Keys et al., 2005). Beside the GRKs, several other protein kinases and phosphatases are implicated in cardiovascular diseases (Anderson et al., 2006; Kacimi and Gerdes, 2003), but these are beyond the scope of this review. In most cases these alterations most likely reflect compensatory mechanism. However, some of these alterations may be the cause of certain pathological aspects of the disease.

GPCR INTERACTING PROTEINS

Many GPCRs interact not only with G proteins but also with accessory proteins called GPCR interacting proteins (GIPs). These GIPs are involved in e.g. GPCR targeting to specific cellular compartments, assembling of large signalling complexes, GPCR trafficking to and from the plasma membrane and fine-tuning of GPCR signalling (Bockaert et al., 2004).

One of the first proteins shown to directly interact with GPCRs was arrestin, which is mainly associated with desensitization and internalization of many GPCRs, as described above. However, arrestins are also scaffolding proteins for several MAPK signalling components, such as raf-1, extracellular signal regulated kinase (ERK), c-Src, Jun N-terminal kinase 3 (JNK3) (DeWire et al., 2007). Unlike many protein-protein interactions, the interactions between arrestin and GPCRs do not appear to depend on a specific binding motif, which explains the scaffolding function for many GPCRs (Moore et al., 2007). Beside interaction with and phosphorylation of GPCRs, GRKs interact with a wide variety of proteins involved in signalling and trafficking, such as $G_{q/11}$, $G\beta\gamma$, PI3 kinase, Akt, MEK1, calmodulin, clathrin, caveolin and actin (reviewed in (Ribas et al., 2007)).

Some GIPs are transmembrane proteins, such as other GPCRs (Marianayagam et al., 2004), ion channels (Fiorentini et al., 2003) and single transmembrane proteins (Hay et al., 2006). Several studies suggest that many GPCRs in all subfamilies are able to form either homodimers or heterodimers with other GPCRs (Marianayagam et al., 2004). Several GPCRs seem to require dimerization for their transport to the plasma membrane (Ng et al., 1999; Salahpour et al., 2004). A well-known example is the heterodimeric $GABA_B$ receptor, which requires both subunits to be a fully functional receptor. The $GABA_BR1$ and $GABA_BR2$ receptors are non-functional as monomers. Only $GABA_BR1$-R2 heterodimers are expressed at the membrane and able to stimulate signalling (Galvez et al., 2001).

Several studies suggest that all aminergic GPCRs are able to form homodimers, but show selectivity in the formation of heterodimers. For instance the α_{1D}-adrenoceptor can form a heterodimer with the α_{1B}- and the β_2-adrenoceptor, but not with the α_{1A}-adrenoceptor (Hague et al., 2006; Uberti et al., 2005). GPCR dimerization may lead to pharmacological profiles distinct from those of the monomeric receptor (Bai, 2004; Breit et al., 2004; Milligan, 2004; Novi et al., 2004; Prinster et al., 2005; Xu et al., 2003; Zhu et al., 2005). However, most of these studies have been performed in overexpression systems in vitro and it is still a debate if receptor dimerization also occurs in vivo.

The intracellular GIPs mainly interact with the C-terminal tail of the GPCR via specific protein-recognition domains, such as PDZ (PSD-95, Disc large, Zonula occludens) domains and domains binding to proline-rich motifs on GPCRs (Bockaert et al., 2004). Since most of the GIPs have multiple protein-protein interaction domains, they form large signalling complexes in association with individual GPCRs, resulting in compartmentalization of receptors and signalling components that enable the regulation of specificity, intensity and duration

of the signalling process. These signalling complexes are thought to be localized to specific membrane lipid compartments, called lipid rafts which are present in every mammalian cell (Ostrom and Insel, 2004). Several GPCRs important in the cardiovascular system have been shown to be localized in or recruited to lipid rafts and/or caveolae, such as the endothelin receptors, the α_1-, β_1- and β_2-adrenoceptors, angiotensin II AT_1 receptor, adenosine A_1 receptor and the M_2 muscarinic receptor (Chini and Parenti, 2004; Insel et al., 2005a; Ostrom and Insel, 2004). GPCR localization in specific lipid compartments seems to be important for GPCR signalling properties, although the exact role of these compartments in GPCR regulation is still unknown.

Box 1. Some specific GIPs in the cardiovascular system

Well known GIPs for the β_2-adrenoceptor are two members of the A-kinase anchoring protein (AKAP) family, AKAP79 and AKAP250 (or Gravin), which are able to interact with PKA, PKC and protein phosphatase 2B and thus function as scaffold protein (Appert-Collin et al., 2006; Wang et al., 2006). Following stimulation of the β_2-adrenoceptor, PKA anchored to AKAP79, is activated and phosphorylates the third intracellular loop of the receptor. This phosphorylation causes the receptor to switch from G_s coupling to G_i coupling and promotes MAPK signalling. By facilition of this G protein switch AKAP79 also attenuates the positive inotropic effects of the receptor. In addition, anchored PKA also phosphorylates GRK2 resulting in increased desensitization of the β_2-adrenoceptor (Appert-Collin et al., 2006). Interaction of AKAP250 with the β_2-adrenoceptor was shown to be important in the desensitization as well as the resensitization and recycling of the receptor (Lin et al., 2000).

Another GIP also interacting with the β_2-adrenoceptor is the Na^+/H^+ exchanger-regulating factor 1 (NHERF1 or EBP250) via its PDZ domain. This direct interaction, which is stimulated by GRK5-mediated receptor phosphorylation, enables the regulation of Na^+/H^+ exchange by the β_2-adrenoceptor without G protein activation (Hall et al., 1998a; Hall et al., 1998b). Direct interaction with NHERF1 also mediates recycling of internalized β_2-adrenoceptors to the membrane (Cao et al., 1999).

An important GIP for the angiotensin II AT_1 receptor is the AT_1 receptor-associated protein (ATRAP), which binds to the C-terminal tail of the receptor (Daviet et al., 1999). ATRAP could act as negative regulator in AT_1-mediated cell proliferation and vascular remodeling by enhancement of AT_1 receptor internalization and down-regulation (Azuma et al., 2007; Oshita et al., 2006). By controlling AT_1 receptor internalization, ATRAP may play a role in the prevention of Angiotensin II-induced pathophysiological conditions, such as cardiac hypertrophy (Tanaka et al., 2005).

Several GIPs, such as Homer proteins (Brakeman et al., 1997), GIPC (GAIP (Gα-interacting protein) interacting protein, C terminus) (Jeanneteau et al., 2004), 14-3-3 proteins (Prezeau et al., 1999), calmodulin (Turner and Raymond, 2005) and spinophilin (Liu et al., 2006; Richman et al., 2001; Smith et al., 1999) were shown to link GPCRs with cytoskeletal proteins or with several effectors ranging from receptors and ion channels to protein kinases and RGS proteins (Wang et al., 2007). These interactions might influence receptor localization and receptor signalling (Hall and Lefkowitz, 2002). Some GIPs might interact with several receptors, such as GRK and arrestin, whereas other interactions are very specific for one GPCR, such as the AT_1 receptor-associated protein (ATRAP). Several interactions between GPCRs and GIPs have been found to be enhanced by agonist stimulation, e.g. GRK, arrestin, NHERF1 interaction, but for many interactions agonist regulation is still unknown. The large number and variety of GIPs as well as their GPCR selectivity further complicates GPCR signalling in physiological and pathophysiological conditions. In Box 1 a limited amount of specific GIP-GPCR interactions are described, which are physiologically relevant in the cardiovascular system.

Another important family of GIPs interacting with several GPCRs and regulating their signalling is the RGS protein family, which is described in detail below.

RGS PROTEINS

RGS proteins are important proteins regulating GPCR-induced signalling by enhancing GTP hydrolysis, thereby terminating the G protein activation cycle (Fig. 2). RGS proteins are defined by a shared 120-130 amino acid domain (the RGS domain) that binds directly to activated Gα subunits to enhance its intrinsic GTPase activity with a minimum of 40-fold over basal levels (Berman et al., 1996b; Hollinger and Hepler, 2002). The RGS domain exhibit highest affinity for the GDP-Mg^{2+}-AlF^{4-} bound Gα, which mimics the transition state during GTP hydrolysis (Berman et al., 1996a; Ross and Wilkie, 2000). More than 30 RGS and RGS-like (containing a RGS-homology domain) proteins have been identified which are divided into six distinct subfamilies based on amino acid sequence, overall protein structure and identified functions within subfamilies. These include the A/RZ (RGS17, 19 (or GAIP), 20, Ret-RGS1), B/R4 (RGS1-5, 8, 13, 16, 18, 21), C/R7 (RGS6, 7, 9, 11), D/R12 (RGS10, 12, 14), E/RA (Axin, Conductin) and F/RL (RGS-like proteins such as RhoGEFs, GRKs, AKAPs and sorting nexins (SNXs)) subfamilies (Ross and Wilkie, 2000; Siderovski and Willard, 2005).

Members of the C/R7, D/R12, E/RA and F/RL subfamilies are large complex proteins (up to 160 kDa) with several additional domains (e.g. Disheveled, EGL-10, Pleckstrin domains, Gγ subunit-like domains, Rap binding domain, GoLoco motifs, phosphotyrosine binding and PDZ domains) allowing interaction with various signalling proteins. RGS proteins of the A/RZ and B/R4 subfamilies are small proteins (20-30 kDa) with a short N-terminal region and a

C-terminal RGS domain, with the exception of RGS3 which has a relatively long N-terminal part of ~300 amino acids (Ross and Wilkie, 2000; Siderovski and Willard, 2005).

For RGS2, RGS3 and RGS5 alternative splice variants were found, that may have different specific functions (Gu et al., 2008; Liang et al., 2005; Mittmann et al., 2001).

GAP activity

RGS proteins were initially characterized by their GAP activity for Gα subunits, thereby increasing G protein inactivation (see Fig. 2). However, no GAP activity was shown for the RGS proteins of the E/RA family and the RGS-like proteins, except the RhoGEFs (Hollinger and Hepler, 2002).

Because of their GAP activity RGS proteins might in general serve as inhibitors of G protein signalling. However, some studies show an enhancement of GPCR signalling by RGS4 proteins (Boutet-Robinet et al., 2003; Zhong et al., 2003). The enhancing effect of RGS4 was

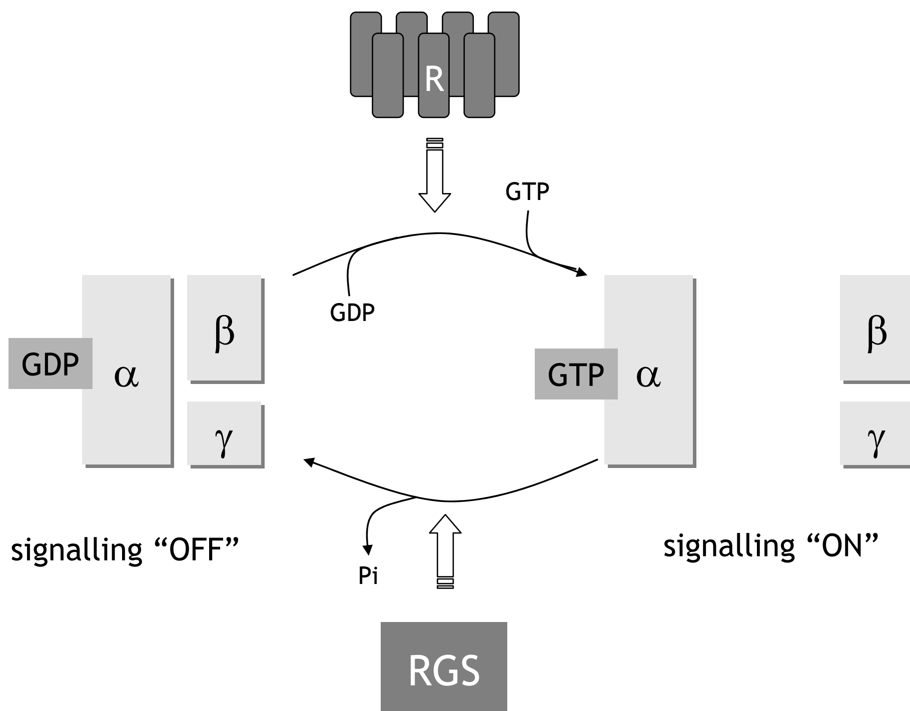

Fig. 2. Effect of RGS proteins on the G protein activation cycle. A GPCR activates the G protein by enhancing GDP-GTP exchange on the Gα-subunit. Gα and Gβγ subunits dissociate and stimulate their respective effectors (signalling "ON"). RGS proteins serve as GTPase activating proteins that enhance GTP hydrolysis and thereby inactivate the Gα-subunit, followed by reassociation with the Gβγ subunit.

Table 1. GPCRs expressed in the cardiovascular system with their interacting G proteins and functional effects of RGS proteins.

GPCR	G protein coupling	Effective RGS	Ineffective RGS	Functional effect	Cell type
Angiotensin II AT_1	$G_{q/11}$ (G_i)	RGS1, RGS2, RGS3L, RGS4[1]		MAPK activity ↓	HeLa
		RGS5[2]	RGS2, RGS3, RGS7[2]	MAPK activity ↓	VSMC
		RGS5[2]	RGS3[2]	IP formation ↓	VSMC
		RGS2[3]	RGS4[3]	Inhibition of cAMP formation ↓	VSMC
		RGS2[4]		IP formation ↓	HEK293T
		RGS4, RGS5[5]		Ca^{2+} response ↓	HEK293T
α_{1A}-adrenoceptor	$G_{q/11}$	RGS2[6]	RGS16[6]	IP formation ↓	HEK293
α_{1B}-adrenoceptor	$G_{q/11}$	nd	RGS2[6]	IP formation ↓	HEK293
α_{1X}-adrenoceptor		RGS2, RGS4[14]		MAPK activity ↓	ARVM
		RGS2[15]		MAPK activity ↓, JNK activity	Cardiomyocytes
		RGS2[16,17], RGS3, RGS4, RGS5[17]		IP formation ↓	Cardiomyocytes
			RGS2[16]	MAPK activity ↓	Cardiomyocytes
β_2-adrenoceptor	G_s (G_i)	RGS2[7]	nd	cAMP formation ↓	HEK293
Endothelin ET_A	$G_{q/11}$ (G_i)	RGS3L, RGS4[1]	RGS1, RGS2[1]	MAPK activity ↓	HeLa
		RGS4, RGS5[5]		Ca^{2+} response ↓	HEK293T
Endothelin ET_X		RGS2, RGS4[14]		MAPK activity ↓	ARVM
		RGS2[16,17], RGS3, RGS4, RGS5[17]		IP formation ↓	Cardiomyocytes
			RGS2[16]	MAPK activity ↓	Cardiomyocytes
Muscarinic M_1	$G_{q/11}$	RGS2, RGS3, RGS8[4]	RGS4, RGS5[4]	Ca^{2+} response ↓	HEK293T
		RGS2, RGS8[4]		IP formation ↓	HEK293T
		RGS2, RGS16[8]		IP formation ↓	CHO
		RGS2, RGS5[9]		IP formation ↓, Ca^{2+} response ↓	COS-7, HEK293

Muscarinic M_2	G_i	RGS1, RGS3, RGS4[10]	RGS2[10]	GIRK current	Oocytes, CHO
		RGS3s, RGS3, RGS4, RGS5, RGS16[13]	RGS2[13]	MAPK activity ↓	COS-7
		RGS2, RGS3s, RGS3, RGS4, RGS5, RGS16[13]		AKT activity ↓	COS-7
		RGS3, RGS4, RGS5[17]	RGS2[17]	Inhibition of cAMP formation ↓	Cardiomyocytes
Muscarinic M_3	$G_{q/11}$	RGS3[2]	RGS2, RGS5, RGS7[2]	MAPK activity ↓	VSMC
		RGS2, RGS3, RGS8[4]	RGS4, RGS5[4]	Ca^{2+} response ↓	HEK293T
		RGS2, RGS8[4]		IP formation ↓	HEK293T
		RGS2, RGS3, RGS5, RGS16[11,13]	RGS4[13]	IP formation ↓	COS-7
		RGS2, RGS3[12]	RGS4[12]	IP formation ↓	HEK293
		RGS2, RGS3, RGS412		Ca^{2+} response ↓	HEK293
		RGS2, RGS3s, RGS3, RGS5[13]	RGS4, RGS16[13]	MAPK activity ↓	COS-7
		RGS3s, RGS3[13]	RGS2, RGS4, RGS5, RGS16[13]	AKT activity ↓	COS-7
Muscarinic M_5	$G_{q/11}$	RGS2, RGS3, RGS8[4]	RGS4, RGS5[4]	Ca^{2+} response ↓	HEK293T
		RGS2, RGS8[4]		IP formation ↓	HEK293T
Sphingosine-1-phosphate $S1P_1$	G_i	RGS1, RGS3L, RGS4[1]	RGS2[1]	MAPK activity ↓	HEK293
Sphingosine-1-phosphate $S1P_2$	G_i, $G_{q/11}$, $G_{12/13}$	RGS1, RGS3L, RGS4[1]	RGS2[1]	MAPK activity ↓	HEK293
Sphingosine-1-phosphate $S1P_3$	G_i, $G_{q/11}$, $G_{12/13}$	RGS1, RGS2, RGS3L[1]	RGS4[1]	MAPK activity ↓	HEK293

nd: not determined, VSMC: vascular smooth muscle cell, HEK: human embryonic kidney, ARVM: adult rat ventricular myocytes, CHO: Chinese hamster ovary, COS-7: African green monkey kidney. [1](Cho et al., 2003a), [2](Wang et al., 2002), [3](Li et al., 2005), [4](Bodenstein et al., 2007), [5](Zhou et al., 2001), [6](Hague et al., 2005), [7](Roy et al., 2006), [8](Bernstein et al., 2004), [9](Gu et al., 2007), [10](Doupnik et al., 1997), [11](Anger et al., 2004), [12](Tovey and Willars, 2004), [13](Anger et al., 2007), [14](Snabaitis et al., 2005), [15](Zou et al., 2006), [16](Zhang et al., 2006), [17](Hao et al., 2006)

shown to be dependent on receptor density and the amount of GTP (Zhong et al., 2003). This enhancement of GPCR signalling might be explained by the increase of the G protein activation-deactivation cycle rate, e.g. the frequency that the G protein is activated by the GPCR (Fig. 2) (Ross and Wilkie, 2000), thereby preventing a rate limiting depletion of GDP-bound G proteins (Zhong et al., 2003). Therefore, the small RGS proteins act as modulators rather than inhibitors of G protein signalling. The larger RGS family members are multifunctional proteins able to form multiprotein complexes and thus act as integrators of G protein signalling by e.g. facilitation of specific signalling pathways (Hollinger and Hepler, 2002).

Most RGS proteins have been shown to act as GAPs for $G\alpha_{i/o}$ and $G\alpha_{q/11}$ proteins (Hollinger and Hepler, 2002). To date only RGS-PX1 has been found to show GAP activity towards $G\alpha_s$ (Zheng et al., 2001) but this finding remains to be confirmed by independent laboratories. The RhoGEFs are the only RGS-like proteins with GAP activity for $G\alpha_{12/13}$ proteins.

There is increasing evidence that RGS proteins are not only able to discriminate between $G\alpha$ subfamilies but that they are also able to discriminate between GPCRs coupled to the same $G\alpha$ subfamily to selectively regulate signalling (Cho et al., 2003a; Ghavami et al., 2004; Wang et al., 2002; Xie and Palmer, 2007; Xu et al., 1999). For example, $G_{q/11}$ signalling of the angiotensin II AT_1 and the endothelin ET_A receptor can be attenuated by RGS5, whereas signalling of the $G_{q/11}$-coupled muscarinic M_3 receptor is not affected (Zhou et al., 2001). As shown in table 1 several RGS proteins selectively regulate GPCR signalling important in the cardiovascular system.

The N-terminal region of small RGS proteins has been shown to be critical for conferring receptor selectivity (Bernstein et al., 2004; Itoh et al., 2006; Zeng et al., 1998). Direct and selective interactions of the N-terminus of several small RGS proteins with the third intracel-

Table 2. Direct interactions of several GPCRs with RGS proteins, determined by pull-down assays with recombinant protein fragments.

GPCR	Direct interacting RGS	No interaction
α_{1A}-adrenoceptor (i3 loop)[1]	RGS2	RGS16
α_{1B}-adrenoceptor (i3 loop)[1]		RGS2, RGS16
α_{1D}-adrenoceptor (i3 loop)[1]		RGS2, RGS16
β_2-adrenoceptor (i3 loop)[2]	RGS2	RGS16
CXCR2 (C-term)[3]	RGS12	
M_1 muscarinic (i3 loop)[2,4,5]	RGS2, RGS4, RGS8	RGS1, RGS16
M_2 muscarinic (i3 loop)[2,4,5]		RGS2, RGS8, RGS16
M_3 muscarinic (i3 loop)[4,5]	RGS2, RGS8	RGS1, RGS4, RGS16
M_4 muscarinic (i3 loop)[4]		RGS1, RGS2, RGS4, RGS16
M_5 muscarinic (i3 loop)[4]	RGS2, RGS4	RGS1, RGS16
δ-opioid (i3 loop and C-term)[6]	RGS4	
μ-opioid (C-term)[6]	RGS4	

[1](Hague et al., 2005), [2](Roy et al., 2006), [3](Snow et al., 1998), [4](Bernstein et al., 2004), [5](Itoh et al., 2006), [6](Georgoussi et al., 2006)

lular loop or the C-terminal tail of GPCRs have been found by using recombinant protein pull-down assays with receptor fragments (see table 2). RGS2 was found to interact directly with a protein fragment of the third intracellular loop of the α_{1A}- but not of the α_{1B}- or α_{1D}-adrenoceptor in a pull-down assay (Hague et al., 2005). Three amino acids (Lys219, Ser220 and Arg238) were shown to be essential for the interaction of the third intracellular (i3) loop of the α_{1A}-adrenoceptor with RGS2 since mutation abolished this interaction (Hague et al., 2005). In addition, mutated α_{1A}-adrenoceptors were insensitive to RGS2 inhibition of functional responses. These amino acids were different from those at the same position in the i3 loop of the α_{1B}-adrenoceptor, explaining the lack of RGS2 interaction of the α_{1B}-adrenoceptor (Hague et al., 2005).

The large RGS proteins might interact directly with the GPCR by one of their protein interaction domains, e.g. the PDZ domain of RGS12 has been found to bind to a PDZ binding motif on the C-terminal tail of the CXC chemokine receptor 2 (CXCR2) (Snow et al., 1998).

Further evidence that RGS proteins are able to interact with GPCRs is the recruitment of RGS proteins to the membrane upon co-expression of certain GPCRs in the absence of agonist stimulation (Roy et al., 2003). The $G_{q/11}$-coupled α_{1A}-adrenoceptor (Hague et al., 2005) and angiotensin II AT_1 receptor and G_s-coupled β_2-adrenoceptor (Roy et al., 2003) are able to recruit RGS2 whereas the G_i-coupled muscarinic M_2 receptors cannot (Roy et al., 2003). In contrast, RGS4 is translocated to the membrane by M_2 but not by AT_1 receptors (Roy et al., 2003).

Other RGS functions

Several RGS proteins have been shown to impair GPCR signalling even in the presence of non-hydrolysable G proteins (Melliti et al., 2001; Sinnarajah et al., 2001), indicating a GAP-independent regulation of GPCR signalling. In some cases, RGS proteins, especially from the B/R4 subfamily, can act as effector antagonists, either by binding to the effector protein or the $G\alpha$ subunit thereby preventing the $G\alpha$-effector interaction. For example, a number of RGS proteins (RGS1, RGS2, RGS3, RGS4, RGS10 and RGS13) are shown to directly inhibit the activity of several AC isoforms (Ghavami et al., 2004; Johnson and Druey, 2002; Roy et al., 2006; Salim et al., 2003; Sinnarajah et al., 2001). For the interaction between RGS2 and AC and its inhibition the N-terminal 19 amino acids of RGS2 were essential whereas the RGS domain was redundant (Salim et al., 2003). In addition, RGS2 was shown to interact with G_s, which might at least partly explain the observed inhibitory effects on cAMP accumulation (Roy et al., 2006). RGS2, RGS3, and RGS4 have been shown to inhibit PLCβ activity induced by GTPγS-activated $G_{q/11}$ proteins (Anger et al., 2004; Cunningham et al., 2001; Hepler et al., 1997; Scheschonka et al., 2000), indicating a GAP-independent attenuation of signalling. This can be explained by inhibition of the $G\alpha_{q/11}$-PLCβ interaction by competitively binding to $G\alpha_{q/11}$. However, RGS4 was shown to interact directly with both $G\alpha_q$ and PLCβ, suggesting a signalling complex in which RGS4 can rapidly attenuate the $G_{q/11}$ signalling (Dowal et al.,

2001). Beside effector antagonism on Gα, also G$\beta\gamma$-mediated signals, such as G$\beta\gamma$-induced PLC, MAPK, PI3kinase activity, can be inhibited directly by RGS proteins, e.g. RGS3 which is able to interact with G$\beta\gamma$ (Shi et al., 2001). Also RGS-like proteins are able to act as effector antagonist, e.g. GRK2 can bind G$\alpha_{q/11}$ via its RGS domain thereby inhibiting $G_{q/11}$ signalling (Carman et al., 1999).

In addition, several small RGS proteins have been found to interact directly with the GIRK channels thereby modulating the G protein-dependent GIRK channel gating kinetics (Doupnik et al., 1997; Herlitze et al., 1999; Inanobe et al., 2001; Kurachi and Ishii, 2004). This modulation might be dependent as well as independent of RGS-GAP activity (Jaen and Doupnik, 2006; Jeong and Ikeda, 2001). RGS4 was shown to accelerate the muscarinic M_2 receptor-mediated GIRK channel 'on' and 'off' kinetics, resulting in a decrease in heart rate (Jaen and Doupnik, 2005; 2006). Also calcium channel activity can be regulated by several RGS proteins, e.g. RGS2 is able to reduce Na^+ and Ca^{2+} currents of the epithelial Ca^{2+} channel TRPV6 by direct interaction with its N-terminus (Schoeber et al., 2006). The exact mechanism of modulation of channel activity is still unknown since 'on' and 'off' rates are modulated differentially by RGS proteins (Bansal et al., 2007).

Regulation of RGS function

Beside interaction with G proteins, GPCRs and some effector proteins, RGS proteins have been found to interact with other proteins influencing their localization, function and stability. For example, interactions of RGS proteins with 14-3-3 proteins (Abramow-Newerly et al., 2006a), Gβ_5 (Rose et al., 2000), Ca^{2+}/calmodulin, PKG1-α (Abramow-Newerly et al., 2006b; Bansal et al., 2007), spinophilin and neurabin (Wang et al., 2007) have been found depending on the RGS protein. Binding of spinophilin to RGS2 promotes the interaction between GPCR, Gα and RGS, thereby enhancing the inhibitory effect of RGS2 on G protein signalling (Wang et al., 2007; Wang et al., 2005). In contrast, binding of neurabin to RGS2 reduces the inhibitory effect of RGS2 on G protein signalling. This can be explained by the inability of neurabin to bind specific GPCRs whereas spinophilin is able to interact directly with the third intracellular loop of several GPCRs (Richman et al., 2001; Smith et al., 1999). Thus the spinophilin/neurabin balance might be important for the inhibitory effect of several RGS proteins on GPCR signalling (Wang et al., 2007).

In addition to regulation by protein interaction, a number of RGS protein functions are known to be regulated by a variety of post-translational modifications such as palmitoylation and phosphorylation (Hollinger and Hepler, 2002; Jones, 2004). Several RGS proteins can be palmitoylated near their N-terminus and at a highly conserved cysteine residue in the RGS domain (Hiol et al., 2003; Osterhout et al., 2003). Palmitoylation near the N-terminus might be involved in membrane association of several RGS proteins, especially the small RGS proteins, although it is not required for all RGS proteins. Palmitoylation in the RGS domain

can either inhibit or potentiate the GAP activity of the RGS protein, depending on their cellular localization (Jones, 2004). In addition, many RGS proteins can be phosphorylated which can either enhance or inhibit GAP activity, dependent on the RGS and the protein kinase involved (Derrien et al., 2003; Hollinger and Hepler, 2002; Moroi et al., 2007). GAP activity of RGS2 towards $G\alpha_{q/11}$ is increased upon phosphorylation by activated PKG1-α, resulting in a decrease of $G\alpha_{q/11}$-mediated contraction in vascular smooth muscle cells (Osei-Owusu et al., 2007; Sun et al., 2005; Tang et al., 2003). Phosphorylation of RGS proteins can also affect interactions with other proteins, e.g. phosphorylation seem to be required for interaction of some RGS proteins with 14-3-3 proteins (Abramow-Newerly et al., 2006a; Bansal et al., 2007).

The membrane phospholipids, phosphatidic acid and phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3), were shown to interact with RGS4 and inhibit its GAP activity (Ishii et al., 2005; Ishii et al., 2002; Ouyang et al., 2003; Popov et al., 2000). PIP_3 inhibition of GAP activity was reversed by Ca^{2+}/calmodulin, which competes for the same interaction site in the RGS domain of RGS4 (Ishii et al., 2005). The reciprocal regulation by PIP_3 and Ca^{2+}/calmodulin may be important for the control of several RGS proteins, since the interaction site is conserved in different RGS proteins (Popov et al., 2000). The physiological importance of PIP_3 regulation of RGS activity was demonstrated by analysis of muscarinic M_2 receptor-mediated GIRK channel activity in cardiomyocytes by whole-cell patch-clamp technique. PIP_3 blocked the effect of RGS4 on GIRK channel activity, which was reversed by Ca^{2+}/calmodulin (Ishii et al., 2002).

Regulation of RGS expression levels

The mRNA levels of different RGS proteins are known to be regulated by a variety of stimuli, including GPCR agonists (Benzing et al., 1999; Berger et al., 2005; Panetta et al., 1999; Patten et al., 2002; Taymans et al., 2004; Zou et al., 2006). This regulation is both tissue- and receptor-specific. Up-regulation of RGS expression might be very fast, for instance RGS2 mRNA and protein expression is up-regulated in response to angiotensin II stimulation within one hour (Grant et al., 2000; Li et al., 2005). An increase in RGS levels in response to the stimulation of a given GPCR might serve as a negative feedback loop to limit signalling responses to the GPCR itself, but it may also lead to decreased responsiveness for other GPCRs. Since several ligands for other types of receptors, such as the receptor tyrosine kinases, can also regulate RGS expression levels, RGS up-regulation may play a role in the cross-talk between GPCRs and other types of receptors. For example RGS1 mRNA, RGS4 protein and RGS16 mRNA and protein up-regulation was demonstrated after endotoxin exposure in rat and pig heart and RGS16 was also up-regulated in cells after exposure to epidermal growth factor (Derrien et al., 2003; Panetta et al., 1999; Patten et al., 2002). RGS expression might also be down-regulated after stimulation, e.g. RGS4 mRNA and protein is down-regulated in rat forebrain after amphetamine administration (Schwendt et al., 2006).

Given that an increase in RGS protein levels has profound effects on GPCR signalling and physiological responses, removal of the stimuli leading to increased RGS expression will require a reduction in the levels of RGS proteins in order to return to baseline. Experiments, in which protein synthesis was blocked, were used to show that RGS4 has a short half-life of just less than one hour. Degradation of RGS4, RGS5 and RGS16 can occur via the N-end rule pathway mediated by ubiquitin (Bodenstein et al., 2007; Hu et al., 2005; Lee et al., 2005). This pathway involves selective degradation of proteins based on the amino acid at the second position of the N-terminus. Proteins having Asp, Glu or Cys residues at that position can be degraded after the addition of an arginine at their N-terminus by the Ate1-encoded arginine transferase. Ate1 knockout in animals leads to lethal cardiovascular defects, indicating that degradation of these N-end pathway substrates is required for normal development (Kwon et al., 2002).

RGS PROTEINS IN THE CARDIOVASCULAR SYSTEM

Gene expression of virtually all RGS proteins has been detected in the mammalian heart as well as in cultured cardiomyocytes, although protein levels might be very low (Doupnik et al., 2001; Kardestuncer et al., 1998; Mittmann et al., 2002; Mittmann et al., 2001; Patten et al., 2002; Riddle et al., 2005; Wieland and Mittmann, 2003). In the human heart RGS expression was mostly studied in the left ventricular myocardium or in whole heart. In human atrium only the two splice variants of RGS5 were detected (Adams et al., 2000). In human myocardium high expression of several members of the B/R4 subfamily was detected, such as RGS2, RGS3, RGS4 and RGS5. Also RGS6, RGS9, RGS11, RGS19 and p115RhoGEF were detected in the human myocardium (Wieland and Mittmann, 2003).

Also in the vascular system gene expression of several RGS proteins was shown with differences between vessels and vascular cell type (Adams et al., 2000; Bondjers et al., 2003; Cho et al., 2003a; Cho et al., 2003b; Grant et al., 2000; Heximer et al., 2003; Horrevoets et al., 1999; Wang et al., 2002). For example RGS5 is highly expressed in the aorta (Adams et al., 2000) and peripheral arteries (Li et al., 2004), especially in pericytes and medial smooth muscle cells (Bondjers et al., 2003; Cho et al., 2003b), whereas in the human vena cava (Adams et al., 2000), peripheral veins and coronary arteries (Li et al., 2004) RGS5 expression was hardly detectable. Therefore, RGS5 was thought to be a specific marker for peripheral arterial smooth mucle cells. However, in human umbilical vein endothelial cells RGS5 expression was detected (Horrevoets et al., 1999). This finding is in contradiction with the specific arterial expression of RGS5, but umbilical vessels are known to be different from peripheral vessels in several ways.

The pericyte-specific RGS5 expression most likely indicate the involvement of RGS5 in vascular maturation and remodelling by inhibiting both PDGF and GPCR-mediated MAPK signalling and thus inhibition of migration (Berger et al., 2005; Bondjers et al., 2003; Cho et al.,

2003b; Mitchell et al., 2008). This might also explain the up-regulation of RGS5 mRNA in the vasculature of several tumours (Berger et al., 2005; Furuya et al., 2004). As some RGS3 splice variants are specifically expressed in endothelial cells (Cho et al., 2003a) and RGS3 mRNA expression is induced during endothelial morphogenesis (Bell et al., 2001), RGS3 might be involved in angiogenesis by inhibiting GPCR-mediated migration of the endothelial cells, although the exact mechanism is still unknown. Besides RGS3 there are indications that RGS4 might be involved in angiogenesis as was shown by inhibiting MAPK and vascular endothelial growth factor signalling in a tube formation assay (Albig and Schiemann, 2005).

The importance of RGS proteins in cardiovascular function was shown by using specific RGS knock-out mice or using RGS-insensitive G protein knock-in cells or mice (Fu et al., 2004).

RGS2 was shown to be crucial in the regulation of vascular tone as a deficiency in RGS2 expression leads to severe hypertension and a prolonged vasoconstriction in animal models, especially in response to angiotensin II (Heximer et al., 2003; Sun et al., 2005; Tang et al., 2003). In general, by inhibiting $G_{q/11}$-mediated signalling RGS2 attenuates contraction in vascular smooth muscle cells.

Muscarinic M_2 and adenosine A_1 receptor-mediated negative chronotropic effects were enhanced by knock-in of RGS-insensitive $G\alpha_o$ (Fu et al., 2006). In addition, knock-in of RGS-insensitive $G\alpha_{i2}$ enhanced muscarinic M_2 but not adenosine A_1 receptor-mediated negative chronotropic effects, whereas β_2-adrenoceptor-mediated positive chronotropic effects were abolished (Fu et al., 2007; Fu et al., 2006).

Altered RGS expression in cardiovascular disease

Alterations in RGS levels have been associated with a number of pathophysiological conditions as well as genetic syndromes. As describe before, RGS2 seems to be crucial in the regulation of vascular tone as RGS2 knockout mice are hypertensive and show a prolonged vasoconstriction in response to angiotensin II (Heximer et al., 2003). The important role of RGS2 was confirmed by a study on patients with Bartter's/Gitelman's syndrome, which is characterized by hypotension and several other defects (Calo et al., 2004). Cells from these patients showed increased mRNA and protein expression levels of RGS2, which may lead to increased NO-mediated relaxation (Sun et al., 2005; Tang et al., 2003). In contrast, a decrease in RGS2 mRNA and protein levels was shown in cells from hypertensive patients (Semplicini et al., 2006; Yang et al., 2005).

Gene expression studies in stroke-prone spontaneously hypertensive rats showed a down-regulation of RGS5 in cerebral microcapillaries (Kirsch et al., 2001) and an increase of RhoGEF mRNA in the aorta (Ying et al., 2004). This might suggest the involvement of RGS5 and Rho-GEF in hypertension although the mechanisms are still unknown. Probably, the effect of RGS5 on migratory response may contribute to the observed phenotype.

RGS5 mRNA was down-regulated in atherosclerosis, especially in the migrated smooth muscle cells in the atherosclerotic plaque and fibrous cap (Adams et al., 2006; Li et al., 2004). Also in the endothelial cells a decrease in RGS5 mRNA expression was found during the inflammatory reaction of atherosclerotic lesions (Horrevoets et al., 1999). In addition, a decrease in RGS5 mRNA expression was associated with neointima formation (Geary et al., 2002). The loss of RGS5 may result in decreased inhibition of hypertrophic responses, such as $G_{q/11}$-mediated MAPK signalling. However, the exact role of RGS5 in atherosclerosis still has to be elucidated.

In cardiac hypertrophy and heart failure expression profiles of RGS2, RGS3 and RGS4 are changed, although inconsistent results were obtained in human as well as in animal studies (Mittmann et al., 2002; Owen et al., 2001; Takeishi et al., 2000; Wieland and Mittmann, 2003; Zhang et al., 2006). In the human failing left ventricular myocardium an increase in RGS4 mRNA and protein was shown (Mittmann et al., 2002; Owen et al., 2001), whereas RGS2 expression was decreased (Takeishi et al., 2000) or not changed (Mittmann et al., 2002). RGS3 expression was reported to increase moderately in human heart failure (Owen et al., 2001), but others showed no change in RGS3 expression levels (Takeishi et al., 2000). Decreased RGS2 expression, which already occurred prior to hypertrophy, might lead to increased $G_{q/11}$-mediated signalling resulting in hypertrophy (Zhang et al., 2006; Zou et al., 2006). Mice overexpressing RGS4 were not able to compensate a pressure overload with hypertrophic mechanisms of the heart possibly due to the attenuation of $G_{q/11}$ signalling, resulting in an increased mortality after pressure overload (Rogers et al., 1999). However, in mice overexpressing G_q an increase in RGS4 expression was beneficial in cardiomyopathy (Rogers et al., 1999). This is possibly due to attenuation of G_q signalling in the heart, but the exact role of the RGS proteins in hypertrophy and heart failure is still unknown.

In animal models for sepsis using the bacterial endotoxin lipopolysaccharide changes in myocardial RGS expression were shown. In pigs an increase in RGS1 and RGS16 mRNA was found in the myocardium as well as the aorta (Panetta et al., 1999). However, in rat myocardium RGS1 mRNA expression was not changed during sepsis, whereas lipopolysaccharide induced an up-regulation of RGS4 and RGS16 protein (Patten et al., 2002). Up-regulation of RGS4 and RGS16 result in decreased $G_{q/11}$-mediated PLC activity, which might explain the onset of heart failure during sepsis (Patten et al., 2002). In the vessels up-regulation of these RGS proteins might explain the hyporesponsiveness to vasoconstrictors, which act mainly via $G_{q/11}$-coupled GPCRs, during sepsis.

As alterations in RGS protein expression was detected in a variety of cardiovascular diseases, they can be suggested to play a role in cardiovascular physiology and pathophysiology.

CONCLUSION

GPCR signalling plays a pivotal role in the cardiovascular system. This signalling is tightly regulated on the GPCR level by desensitization/resensitization mechanisms and up-/down-regulation of expression (Fig. 1). The responsiveness of GPCRs is controlled by interactions with GIPs, especially by RGS and RGS-like proteins. RGS proteins play a role in the physiological regulation and in the pathophysiological changes in the cardiovascular system, although it is difficult to get a detailed insight in their role because of the multiple functions of RGS proteins due to the variety of domains. Although RGS proteins can be considered interesting new drug targets in cardiovascular diseases, no drugs interacting with these proteins have been developed yet. This might be due to the limited knowledge about their role in physiology and the pathophysiology of the cardiovascular system. In addition, suitable compounds that intervene with RGS protein function are still missing. Nevertheless, RGS proteins are interesting targets for future drugs in cardiovascular diseases.

AIM OF THE THESIS

GPCRs play a role in many biological processes and form the largest class of drug targets to which ligands are currently directed. Because of the important role in essential physiological function, including cardiovascular functions such as heart rate, heart contractility and vascular tone, the signalling of GPCRs is tightly controlled. Signalling of GPCRs can be regulated in different ways and at different levels. Regulation at the level of the ligand comprises instability, membrane transport and degradation of the ligand which might influence the interaction with the GPCR and hence the GPCR signalling. Regulation at the GPCR level involves desensitization and resensitization processes that change the availability of receptors to be stimulated, and up- and downregulation of receptors, which changes the amount of GPCRs. The responsiveness of GPCRs can be controlled by the regulation of expression, interaction, activity and translocation of signalling proteins such as G proteins, protein kinases, phospholipases and RGS proteins.

S1P receptors represent a recently discovered GPCR family and it is largely unknown how the signalling of the S1P receptors is regulated. The studies described in this thesis were aimed at elucidating some aspects of regulation of S1P receptor signalling and the possible involvement of RGS proteins in regulation of the S1P receptor signalling and of other vasoactive ligands.

At the time this research project was started, no data were available on the influence of S1P stability on S1P receptor signalling as the S1P receptor field is relatively new. Moreover, S1P is known to be degraded by several enzymes (Le Stunff et al., 2004; Pyne and Pyne, 2002) and, as it is a lipid, S1P might easily attach to surfaces. Therefore, we investigated whether the S1P

concentration was stable over time and its impact on S1P receptor signalling, especially on S1P potency (*Chapter 2*).

Internalization was only studied for the $S1P_1$ receptor (Oo et al., 2007) but not for the $S1P_3$ receptor, a receptor involved in the cardiac side-effects of the immunosuppressant pro-drug FTY720. Since it was postulated that internalization of the $S1P_1$ receptor was the mechanism of action of FTY720, we investigated whether S1P and FTY720-P would have similar actions on internalization and subsequent recycling of the $S1P_3$ receptor (*Chapter 3*).

Vascular smooth muscle cells (VSMCs) can change phenotype from contractile to a more synthetic or proliferative phenotype (Hayashi et al., 2001; Sobue et al., 1999). Both S1P and RGS proteins have been implicated in VSMC proliferation and migration and hence may play a role in phenotypic modulation. We used both phenotypic modulation (*Chapter 4*) and direct S1P stimulation (*Chapter 5*) to investigate the effects on expression levels of specific RGS proteins. In addition, alterations in RGS expression levels were used to investigate its effects on S1P receptor signalling (*Chapter 4*).

Finally, RGS protein expression was investigated in a septic shock model to explain the differential impairment of contraction by some vasoactive compounds (*Chapter 6*).

REFERENCES

Abramow-Newerly, M., Ming, H., Chidiac, P., 2006a. Modulation of subfamily B/R4 RGS protein function by 14-3-3 proteins. Cell Signal 18, 2209-2222.

Abramow-Newerly, M., Roy, A.A., Nunn, C., Chidiac, P., 2006b. RGS proteins have a signalling complex: interactions between RGS proteins and GPCRs, effectors, and auxiliary proteins. Cell Signal 18, 579-591.

Adams, L.D., Geary, R.L., Li, J., Rossini, A., Schwartz, S.M., 2006. Expression profiling identifies smooth muscle cell diversity within human intima and plaque fibrous cap: loss of RGS5 distinguishes the cap. Arterioscler Thromb Vasc Biol 26, 319-325.

Adams, L.D., Geary, R.L., McManus, B., Schwartz, S.M., 2000. A comparison of aorta and vena cava medial message expression by cDNA array analysis identifies a set of 68 consistently differentially expressed genes, all in aortic media. Circ Res 87, 623-631.

Albig, A.R., Schiemann, W.P., 2005. Identification and characterization of regulator of G protein signaling 4 (RGS4) as a novel inhibitor of tubulogenesis: RGS4 inhibits mitogen-activated protein kinases and vascular endothelial growth factor signaling. Mol Biol Cell 16, 609-625.

Allen, B.G., Walsh, M.P., 1994. The biochemical basis of the regulation of smooth-muscle contraction. Trends Biochem Sci 19, 362-368.

Anderson, M.E., Higgins, L.S., Schulman, H., 2006. Disease mechanisms and emerging therapies: protein kinases and their inhibitors in myocardial disease. Nat Clin Pract Cardiovasc Med 3, 437-445.

Anger, T., Klintworth, N., Stumpf, C., Daniel, W.G., Mende, U., Garlichs, C.D., 2007. RGS Protein Specificity Towards G(q)- and G(i/o)-Mediated ERK 1/2 and Akt Activation, in vitro. J Biochem Mol Biol 40, 899-910.

Anger, T., Zhang, W., Mende, U., 2004. Differential contribution of GTPase activation and effector antagonism to the inhibitory effect of RGS proteins on Gq-mediated signaling in vivo. J Biol Chem 279, 3906-3915.

Appert-Collin, A., Baisamy, L., Diviani, D., 2006. Regulation of g protein-coupled receptor signaling by a-kinase anchoring proteins. J Recept Signal Transduct Res 26, 631-646.

Azuma, K., Tamura, K., Shigenaga, A., Wakui, H., Masuda, S., Tsurumi-Ikeya, Y., Tanaka, Y., Sakai, M., Matsuda, M., Hashimoto, T., Ishigami, T., Lopez-Ilasaca, M., Umemura, S., 2007. Novel regulatory effect of angiotensin II type 1 receptor-interacting molecule on vascular smooth muscle cells. Hypertension 50, 926-932.

Bai, M., 2004. Dimerization of G-protein-coupled receptors: roles in signal transduction. Cell Signal 16, 175-186.

Baker, J.G., Hill, S.J., 2007. Multiple GPCR conformations and signalling pathways: implications for antagonist affinity estimates. Trends Pharmacol Sci 28, 374-381.

Balmforth, A.J., Shepherd, F.H., Warburton, P., Ball, S.G., 1997. Evidence of an important and direct role for protein kinase C in agonist-induced phosphorylation leading to desensitization of the angiotensin AT1A receptor. Br J Pharmacol 122, 1469-1477.

Bansal, G., Druey, K.M., Xie, Z., 2007. R4 RGS proteins: Regulation of G-protein signaling and beyond. Pharmacol Ther 116, 473-495.

Bell, S.E., Mavila, A., Salazar, R., Bayless, K.J., Kanagala, S., Maxwell, S.A., Davis, G.E., 2001. Differential gene expression during capillary morphogenesis in 3D collagen matrices: regulated expression of genes involved in basement membrane matrix assembly, cell cycle progression, cellular differentiation and G-protein signaling. J Cell Sci 114, 2755-2773.

Benovic, J.L., Pike, L.J., Cerione, R.A., Staniszewski, C., Yoshimasa, T., Codina, J., Caron, M.G., Lefkowitz, R.J., 1985. Phosphorylation of the mammalian beta-adrenergic receptor by cyclic AMP-dependent protein kinase. Regulation of the rate of receptor phosphorylation and dephosphorylation by agonist

occupancy and effects on coupling of the receptor to the stimulatory guanine nucleotide regulatory protein. J Biol Chem 260, 7094-7101.

Benzing, T., Brandes, R., Sellin, L., Schermer, B., Lecker, S., Walz, G., Kim, E., 1999. Upregulation of RGS7 may contribute to tumor necrosis factor-induced changes in central nervous function. Nat Med 5, 913-918.

Berger, M., Bergers, G., Arnold, B., Hammerling, G.J., Ganss, R., 2005. Regulator of G-protein signaling-5 induction in pericytes coincides with active vessel remodeling during neovascularization. Blood 105, 1094-1101.

Berman, D.M., Kozasa, T., Gilman, A.G., 1996a. The GTPase-activating protein RGS4 stabilizes the transition state for nucleotide hydrolysis. J Biol Chem 271, 27209-27212.

Berman, D.M., Wilkie, T.M., Gilman, A.G., 1996b. GAIP and RGS4 are GTPase-activating proteins for the Gi subfamily of G protein alpha subunits. Cell 86, 445-452.

Bernstein, L.S., Ramineni, S., Hague, C., Cladman, W., Chidiac, P., Levey, A.I., Hepler, J.R., 2004. RGS2 binds directly and selectively to the M1 muscarinic acetylcholine receptor third intracellular loop to modulate Gq/11alpha signaling. J Biol Chem 279, 21248-21256.

Bockaert, J., Fagni, L., Dumuis, A., Marin, P., 2004. GPCR interacting proteins (GIP). Pharmacol Ther 103, 203-221.

Bockaert, J., Pin, J.P., 1999. Molecular tinkering of G protein-coupled receptors: an evolutionary success. Embo J 18, 1723-1729.

Bodenstein, J., Sunahara, R.K., Neubig, R.R., 2007. N-terminal residues control proteasomal degradation of RGS2, RGS4, and RGS5 in human embryonic kidney 293 cells. Mol Pharmacol 71, 1040-1050.

Bondjers, C., Kalen, M., Hellstrom, M., Scheidl, S.J., Abramsson, A., Renner, O., Lindahl, P., Cho, H., Kehrl, J., Betsholtz, C., 2003. Transcription profiling of platelet-derived growth factor-B-deficient mouse embryos identifies RGS5 as a novel marker for pericytes and vascular smooth muscle cells. Am J Pathol 162, 721-729.

Boutet-Robinet, E.A., Finana, F., Wurch, T., Pauwels, P.J., De Vries, L., 2003. Endogenous RGS proteins facilitate dopamine D(2S) receptor coupling to G(alphao) proteins and Ca2+ responses in CHO-K1 cells. FEBS Lett 533, 67-71.

Bouvier, M., Collins, S., O'Dowd, B.F., Campbell, P.T., de Blasi, A., Kobilka, B.K., MacGregor, C., Irons, G.P., Caron, M.G., Lefkowitz, R.J., 1989. Two distinct pathways for cAMP-mediated down-regulation of the beta 2-adrenergic receptor. Phosphorylation of the receptor and regulation of its mRNA level. J Biol Chem 264, 16786-16792.

Brakeman, P.R., Lanahan, A.A., O'Brien, R., Roche, K., Barnes, C.A., Huganir, R.L., Worley, P.F., 1997. Homer: a protein that selectively binds metabotropic glutamate receptors. Nature 386, 284-288.

Brattelid, T., Tveit, K., Birkeland, J.A., Sjaastad, I., Qvigstad, E., Krobert, K.A., Hussain, R.I., Skomedal, T., Osnes, J.B., Levy, F.O., 2007. Expression of mRNA encoding G protein-coupled receptors involved in congestive heart failure--a quantitative RT-PCR study and the question of normalisation. Basic Res Cardiol 102, 198-208.

Breit, A., Lagace, M., Bouvier, M., 2004. Hetero-oligomerization between beta2- and beta3-adrenergic receptors generates a beta-adrenergic signaling unit with distinct functional properties. J Biol Chem 279, 28756-28765.

Brodde, O.E., 1991. Pathophysiology of the beta-adrenoceptor system in chronic heart failure: consequences for treatment with agonists, partial agonists or antagonists? Eur Heart J 12 Suppl F, 54-62.

Brodde, O.E., Bruck, H., Leineweber, K., 2006. Cardiac adrenoceptors: physiological and pathophysiological relevance. J Pharmacol Sci 100, 323-337.

Brodde, O.E., Michel, M.C., 1999. Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev 51, 651-690.

Cabrera-Vera, T.M., Vanhauwe, J., Thomas, T.O., Medkova, M., Preininger, A., Mazzoni, M.R., Hamm, H.E., 2003. Insights into G protein structure, function, and regulation. Endocr Rev 24, 765-781.

Calo, L.A., Pagnin, E., Davis, P.A., Sartori, M., Ceolotto, G., Pessina, A.C., Semplicini, A., 2004. Increased expression of regulator of G protein signaling-2 (RGS-2) in Bartter's/Gitelman's syndrome. A role in the control of vascular tone and implication for hypertension. J Clin Endocrinol Metab 89, 4153-4157.

Cao, T.T., Deacon, H.W., Reczek, D., Bretscher, A., von Zastrow, M., 1999. A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. Nature 401, 286-290.

Carman, C.V., Parent, J.L., Day, P.W., Pronin, A.N., Sternweis, P.M., Wedegaertner, P.B., Gilman, A.G., Benovic, J.L., Kozasa, T., 1999. Selective regulation of Galpha(q/11) by an RGS domain in the G protein-coupled receptor kinase, GRK2. J Biol Chem 274, 34483-34492.

Cherezov, V., Rosenbaum, D.M., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T.S., Choi, H.J., Kuhn, P., Weis, W.I., Kobilka, B.K., Stevens, R.C., 2007. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318, 1258-1265.

Chidiac, P., Ross, E.M., 1999. Phospholipase C-beta1 directly accelerates GTP hydrolysis by Galphaq and acceleration is inhibited by Gbeta gamma subunits. J Biol Chem 274, 19639-19643.

Chini, B., Parenti, M., 2004. G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there? J Mol Endocrinol 32, 325-338.

Cho, H., Harrison, K., Schwartz, O., Kehrl, J.H., 2003a. The aorta and heart differentially express RGS (regulators of G-protein signalling) proteins that selectively regulate sphingosine 1-phosphate, angiotensin II and endothelin-1 signalling. Biochem J 371, 973-980.

Cho, H., Kozasa, T., Bondjers, C., Betsholtz, C., Kehrl, J.H., 2003b. Pericyte-specific expression of Rgs5: implications for PDGF and EDG receptor signaling during vascular maturation. Faseb J 17, 440-442.

Claing, A., Perry, S.J., Achiriloaie, M., Walker, J.K., Albanesi, J.P., Lefkowitz, R.J., Premont, R.T., 2000. Multiple endocytic pathways of G protein-coupled receptors delineated by GIT1 sensitivity. Proc Natl Acad Sci U S A 97, 1119-1124.

Conner, D.A., Mathier, M.A., Mortensen, R.M., Christe, M., Vatner, S.F., Seidman, C.E., Seidman, J.G., 1997. beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. Circ Res 81, 1021-1026.

Cunningham, M.L., Waldo, G.L., Hollinger, S., Hepler, J.R., Harden, T.K., 2001. Protein kinase C phosphorylates RGS2 and modulates its capacity for negative regulation of Galpha 11 signaling. J Biol Chem 276, 5438-5444.

Daaka, Y., Luttrell, L.M., Lefkowitz, R.J., 1997a. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 390, 88-91.

Daaka, Y., Pitcher, J.A., Richardson, M., Stoffel, R.H., Robishaw, J.D., Lefkowitz, R.J., 1997b. Receptor and G betagamma isoform-specific interactions with G protein-coupled receptor kinases. Proc Natl Acad Sci U S A 94, 2180-2185.

Dale, L.B., Bhattacharya, M., Seachrist, J.L., Anborgh, P.H., Ferguson, S.S., 2001. Agonist-stimulated and tonic internalization of metabotropic glutamate receptor 1a in human embryonic kidney 293 cells: agonist-stimulated endocytosis is beta-arrestin1 isoform-specific. Mol Pharmacol 60, 1243-1253.

Daviet, L., Lehtonen, J.Y., Tamura, K., Griese, D.P., Horiuchi, M., Dzau, V.J., 1999. Cloning and characterization of ATRAP, a novel protein that interacts with the angiotensin II type 1 receptor. J Biol Chem 274, 17058-17062.

Derrien, A., Zheng, B., Osterhout, J.L., Ma, Y.C., Milligan, G., Farquhar, M.G., Druey, K.M., 2003. Src-mediated RGS16 tyrosine phosphorylation promotes RGS16 stability. J Biol Chem 278, 16107-16116.

Desai, A.N., Salim, S., Standifer, K.M., Eikenburg, D.C., 2006. Involvement of G protein-coupled receptor kinase (GRK) 3 and GRK2 in down-regulation of the alpha2B-adrenoceptor. J Pharmacol Exp Ther 317, 1027-1035.

DeWire, S.M., Ahn, S., Lefkowitz, R.J., Shenoy, S.K., 2007. Beta-arrestins and cell signaling. Annu Rev Physiol 69, 483-510.

Doupnik, C.A., Davidson, N., Lester, H.A., Kofuji, P., 1997. RGS proteins reconstitute the rapid gating kinetics of gbetagamma-activated inwardly rectifying K+ channels. Proc Natl Acad Sci U S A 94, 10461-10466.

Doupnik, C.A., Xu, T., Shinaman, J.M., 2001. Profile of RGS expression in single rat atrial myocytes. Biochim Biophys Acta 1522, 97-107.

Dowal, L., Elliott, J., Popov, S., Wilkie, T.M., Scarlata, S., 2001. Determination of the contact energies between a regulator of G protein signaling and G protein subunits and phospholipase C beta 1. Biochemistry 40, 414-421.

Dzimiri, N., 2002. Receptor crosstalk. Implications for cardiovascular function, disease and therapy. Eur J Biochem 269, 4713-4730.

El-Armouche, A., Zolk, O., Rau, T., Eschenhagen, T., 2003. Inhibitory G-proteins and their role in desensitization of the adenylyl cyclase pathway in heart failure. Cardiovasc Res 60, 478-487.

Ferguson, S.S., 2001. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 53, 1-24.

Fiorentini, C., Gardoni, F., Spano, P., Di Luca, M., Missale, C., 2003. Regulation of dopamine D1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors. J Biol Chem 278, 20196-20202.

Fredriksson, R., Lagerstrom, M.C., Lundin, L.G., Schioth, H.B., 2003. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 63, 1256-1272.

Fu, Y., Huang, X., Piao, L., Lopatin, A.N., Neubig, R.R., 2007. Endogenous RGS proteins modulate SA and AV nodal functions in isolated heart: implications for sick sinus syndrome and AV block. Am J Physiol Heart Circ Physiol 292, H2532-2539.

Fu, Y., Huang, X., Zhong, H., Mortensen, R.M., D'Alecy, L.G., Neubig, R.R., 2006. Endogenous RGS proteins and Galpha subtypes differentially control muscarinic and adenosine-mediated chronotropic effects. Circ Res 98, 659-666.

Fu, Y., Zhong, H., Nanamori, M., Mortensen, R.M., Huang, X., Lan, K., Neubig, R.R., 2004. RGS-insensitive G-protein mutations to study the role of endogenous RGS proteins. Methods Enzymol 389, 229-243.

Furuya, M., Nishiyama, M., Kimura, S., Suyama, T., Naya, Y., Ito, H., Nikaido, T., Ishikura, H., 2004. Expression of regulator of G protein signalling protein 5 (RGS5) in the tumour vasculature of human renal cell carcinoma. J Pathol 203, 551-558.

Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., Prezeau, L., Pin, J.P., 2001. Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. Embo J 20, 2152-2159.

Geary, R.L., Wong, J.M., Rossini, A., Schwartz, S.M., Adams, L.D., 2002. Expression profiling identifies 147 genes contributing to a unique primate neointimal smooth muscle cell phenotype. Arterioscler Thromb Vasc Biol 22, 2010-2016.

Georgoussi, Z., Leontiadis, L., Mazarakou, G., Merkouris, M., Hyde, K., Hamm, H., 2006. Selective interactions between G protein subunits and RGS4 with the C-terminal domains of the mu- and delta-opioid receptors regulate opioid receptor signaling. Cell Signal 18, 771-782.

Gether, U., 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr Rev 21, 90-113.

Ghanouni, P., Gryczynski, Z., Steenhuis, J.J., Lee, T.W., Farrens, D.L., Lakowicz, J.R., Kobilka, B.K., 2001. Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta 2 adrenergic receptor. J Biol Chem 276, 24433-24436.

Ghavami, A., Hunt, R.A., Olsen, M.A., Zhang, J., Smith, D.L., Kalgaonkar, S., Rahman, Z., Young, K.H., 2004. Differential effects of regulator of G protein signaling (RGS) proteins on serotonin 5-HT1A, 5-HT2A, and dopamine D2 receptor-mediated signaling and adenylyl cyclase activity. Cell Signal 16, 711-721.

Grant, S.L., Lassegue, B., Griendling, K.K., Ushio-Fukai, M., Lyons, P.R., Alexander, R.W., 2000. Specific regulation of RGS2 messenger RNA by angiotensin II in cultured vascular smooth muscle cells. Mol Pharmacol 57, 460-467.

Gros, R., Chorazyczewski, J., Meek, M.D., Benovic, J.L., Ferguson, S.S., Feldman, R.D., 2000. G-Protein-coupled receptor kinase activity in hypertension : increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. Hypertension 35, 38-42.

Gross, C.M., Gerbaulet, S., Quensel, C., Kramer, J., Mittelmeier, H.O., Luft, F.C., Dietz, R., 2002. Angiotensin II type 1 receptor expression in human coronary arteries with variable degrees of atherosclerosis. Basic Res Cardiol 97, 327-333.

Gu, S., Anton, A., Salim, S., Blumer, K.J., Dessauer, C.W., Heximer, S.P., 2008. Alternative translation initiation of human regulators of G-protein signaling-2 yields a set of functionally distinct proteins. Mol Pharmacol 73, 1-11.

Gu, S., He, J., Ho, W.T., Ramineni, S., Thal, D.M., Natesh, R., Tesmer, J.J., Hepler, J.R., Heximer, S.P., 2007. Unique hydrophobic extension of the RGS2 amphipathic helix domain imparts increased plasma membrane binding and function relative to other RGS R4/B subfamily members. J Biol Chem 282, 33064-33075.

Hadcock, J.R., Malbon, C.C., 1988. Down-regulation of beta-adrenergic receptors: agonist-induced reduction in receptor mRNA levels. Proc Natl Acad Sci U S A 85, 5021-5025.

Hadcock, J.R., Wang, H.Y., Malbon, C.C., 1989. Agonist-induced destabilization of beta-adrenergic receptor mRNA. Attenuation of glucocorticoid-induced up-regulation of beta-adrenergic receptors. J Biol Chem 264, 19928-19933.

Hague, C., Bernstein, L.S., Ramineni, S., Chen, Z., Minneman, K.P., Hepler, J.R., 2005. Selective inhibition of alpha1A-adrenergic receptor signaling by RGS2 association with the receptor third intracellular loop. J Biol Chem 280, 27289-27295.

Hague, C., Lee, S.E., Chen, Z., Prinster, S.C., Hall, R.A., Minneman, K.P., 2006. Heterodimers of alpha1B- and alpha1D-adrenergic receptors form a single functional entity. Mol Pharmacol 69, 45-55.

Hall, R.A., Lefkowitz, R.J., 2002. Regulation of G protein-coupled receptor signaling by scaffold proteins. Circ Res 91, 672-680.

Hall, R.A., Ostedgaard, L.S., Premont, R.T., Blitzer, J.T., Rahman, N., Welsh, M.J., Lefkowitz, R.J., 1998a. A C-terminal motif found in the beta2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na+/H+ exchanger regulatory factor family of PDZ proteins. Proc Natl Acad Sci U S A 95, 8496-8501.

Hall, R.A., Premont, R.T., Chow, C.W., Blitzer, J.T., Pitcher, J.A., Claing, A., Stoffel, R.H., Barak, L.S., Shenolikar, S., Weinman, E.J., Grinstein, S., Lefkowitz, R.J., 1998b. The beta2-adrenergic receptor interacts with the Na+/H+-exchanger regulatory factor to control Na+/H+ exchange. Nature 392, 626-630.

Hamm, H.E., 1998. The many faces of G protein signaling. J Biol Chem 273, 669-672.

Hao, J., Michalek, C., Zhang, W., Zhu, M., Xu, X., Mende, U., 2006. Regulation of cardiomyocyte signaling by RGS proteins: differential selectivity towards G proteins and susceptibility to regulation. J Mol Cell Cardiol 41, 51-61.

Hay, D.L., Poyner, D.R., Sexton, P.M., 2006. GPCR modulation by RAMPs. Pharmacol Ther 109, 173-197.

Hayashi, K., Takahashi, M., Nishida, W., Yoshida, K., Ohkawa, Y., Kitabatake, A., Aoki, J., Arai, H., Sobue, K., 2001. Phenotypic modulation of vascular smooth muscle cells induced by unsaturated lysophosphatidic acids. Circ Res 89, 251-258.

Hendriks-Balk, M.C., Michel, M.C., Alewijnse, A.E., 2007. Pitfalls in the normalization of real-time polymerase chain reaction data. Basic Res Cardiol 102, 195-197.

Hepler, J.R., Berman, D.M., Gilman, A.G., Kozasa, T., 1997. RGS4 and GAIP are GTPase-activating proteins for Gq alpha and block activation of phospholipase C beta by gamma-thio-GTP-Gq alpha. Proc Natl Acad Sci U S A 94, 428-432.

Herlitze, S., Ruppersberg, J.P., Mark, M.D., 1999. New roles for RGS2, 5 and 8 on the ratio-dependent modulation of recombinant GIRK channels expressed in Xenopus oocytes. J Physiol 517 (Pt 2), 341-352.

Hermans, E., 2003. Biochemical and pharmacological control of the multiplicity of coupling at G-protein-coupled receptors. Pharmacol Ther 99, 25-44.

Heximer, S.P., Knutsen, R.H., Sun, X., Kaltenbronn, K.M., Rhee, M.H., Peng, N., Oliveira-dos-Santos, A., Penninger, J.M., Muslin, A.J., Steinberg, T.H., Wyss, J.M., Mecham, R.P., Blumer, K.J., 2003. Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice. J Clin Invest 111, 445-452.

Hiol, A., Davey, P.C., Osterhout, J.L., Waheed, A.A., Fischer, E.R., Chen, C.K., Milligan, G., Druey, K.M., Jones, T.L., 2003. Palmitoylation regulates regulators of G-protein signaling (RGS) 16 function. I. Mutation of amino-terminal cysteine residues on RGS16 prevents its targeting to lipid rafts and palmitoylation of an internal cysteine residue. J Biol Chem 278, 19301-19308.

Hollinger, S., Hepler, J.R., 2002. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol Rev 54, 527-559.

Horrevoets, A.J., Fontijn, R.D., van Zonneveld, A.J., de Vries, C.J., ten Cate, J.W., Pannekoek, H., 1999. Vascular endothelial genes that are responsive to tumor necrosis factor-alpha in vitro are expressed in atherosclerotic lesions, including inhibitor of apoptosis protein-1, stannin, and two novel genes. Blood 93, 3418-3431.

Hu, R.G., Sheng, J., Qi, X., Xu, Z., Takahashi, T.T., Varshavsky, A., 2005. The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. Nature 437, 981-986.

Iaccarino, G., Koch, W.J., 2003. Transgenic mice targeting the heart unveil G protein-coupled receptor kinases as therapeutic targets. Assay Drug Dev Technol 1, 347-355.

Inanobe, A., Fujita, S., Makino, Y., Matsushita, K., Ishii, M., Chachin, M., Kurachi, Y., 2001. Interaction between the RGS domain of RGS4 with G protein alpha subunits mediates the voltage-dependent relaxation of the G protein-gated potassium channel. J Physiol 535, 133-143.

Insel, P.A., Head, B.P., Ostrom, R.S., Patel, H.H., Swaney, J.S., Tang, C.M., Roth, D.M., 2005a. Caveolae and lipid rafts: G protein-coupled receptor signaling microdomains in cardiac myocytes. Ann N Y Acad Sci 1047, 166-172.

Insel, P.A., Head, B.P., Patel, H.H., Roth, D.M., Bundey, R.A., Swaney, J.S., 2005b. Compartmentation of G-protein-coupled receptors and their signalling components in lipid rafts and caveolae. Biochem Soc Trans 33, 1131-1134.

Ishii, M., Fujita, S., Yamada, M., Hosaka, Y., Kurachi, Y., 2005. Phosphatidylinositol 3,4,5-trisphosphate and Ca2+/calmodulin competitively bind to the regulators of G-protein-signalling (RGS) domain of RGS4 and reciprocally regulate its action. Biochem J 385, 65-73.

Ishii, M., Inanobe, A., Kurachi, Y., 2002. PIP3 inhibition of RGS protein and its reversal by Ca2+/calmodulin mediate voltage-dependent control of the G protein cycle in a cardiac K+ channel. Proc Natl Acad Sci U S A 99, 4325-4330.

Itoh, M., Nagatomo, K., Kubo, Y., Saitoh, O., 2006. Alternative splicing of RGS8 gene changes the binding property to the M1 muscarinic receptor to confer receptor type-specific Gq regulation. J Neurochem 99, 1505-1516.

Jaber, M., Koch, W.J., Rockman, H., Smith, B., Bond, R.A., Sulik, K.K., Ross, J., Jr., Lefkowitz, R.J., Caron, M.G., Giros, B., 1996. Essential role of beta-adrenergic receptor kinase 1 in cardiac development and function. Proc Natl Acad Sci U S A 93, 12974-12979.

Jaen, C., Doupnik, C.A., 2005. Neuronal Kir3.1/Kir3.2a channels coupled to serotonin 1A and muscarinic m2 receptors are differentially modulated by the "short" RGS3 isoform. Neuropharmacology 49, 465-476.

Jaen, C., Doupnik, C.A., 2006. RGS3 and RGS4 differentially associate with G protein-coupled receptor-Kir3 channel signaling complexes revealing two modes of RGS modulation. Precoupling and collision coupling. J Biol Chem 281, 34549-34560.

Jeanneteau, F., Guillin, O., Diaz, J., Griffon, N., Sokoloff, P., 2004. GIPC recruits GAIP (RGS19) to attenuate dopamine D2 receptor signaling. Mol Biol Cell 15, 4926-4937.

Jeong, S.W., Ikeda, S.R., 2001. Differential regulation of G protein-gated inwardly rectifying K(+) channel kinetics by distinct domains of RGS8. J Physiol 535, 335-347.

Johnson, E.N., Druey, K.M., 2002. Functional characterization of the G protein regulator RGS13. J Biol Chem 277, 16768-16774.

Jones, T.L., 2004. Role of palmitoylation in RGS protein function. Methods Enzymol 389, 33-55.

Kacimi, R., Gerdes, A.M., 2003. Alterations in G protein and MAP kinase signaling pathways during cardiac remodeling in hypertension and heart failure. Hypertension 41, 968-977.

Kardestuncer, T., Wu, H., Lim, A.L., Neer, E.J., 1998. Cardiac myocytes express mRNA for ten RGS proteins: changes in RGS mRNA expression in ventricular myocytes and cultured atria. FEBS Lett 438, 285-288.

Kelly, E., Bailey, C.P., Henderson, G., 2008. Agonist-selective mechanisms of GPCR desensitization. Br J Pharmacol 153 Suppl 1, S379-388.

Kenakin, T., 2001. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. Faseb J 15, 598-611.

Kenakin, T., 2002. Recombinant roulette versus the apparent virtues of 'natural' cell receptor systems: receptor genotypes versus phenotypes. Trends Pharmacol Sci 23, 403-404.

Kenakin, T., 2003. Ligand-selective receptor conformations revisited: the promise and the problem. Trends Pharmacol Sci 24, 346-354.

Kenakin, T., 2007. Functional selectivity through protean and biased agonism: who steers the ship? Mol Pharmacol 72, 1393-1401.

Keys, J.R., Zhou, R.H., Harris, D.M., Druckman, C.A., Eckhart, A.D., 2005. Vascular smooth muscle overexpression of G protein-coupled receptor kinase 5 elevates blood pressure, which segregates with sex and is dependent on Gi-mediated signaling. Circulation 112, 1145-1153.

Kirkham, M., Parton, R.G., 2005. Clathrin-independent endocytosis: new insights into caveolae and non-caveolar lipid raft carriers. Biochim Biophys Acta 1746, 349-363.

Kirsch, T., Wellner, M., Luft, F.C., Haller, H., Lippoldt, A., 2001. Altered gene expression in cerebral capillaries of stroke-prone spontaneously hypertensive rats. Brain Res 910, 106-115.

Kobilka, B.K., Deupi, X., 2007. Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 28, 397-406.

Kristiansen, K., 2004. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. Pharmacol Ther 103, 21-80.

Kurachi, Y., Ishii, M., 2004. Cell signal control of the G protein-gated potassium channel and its subcellular localization. J Physiol 554, 285-294.

Kwon, Y.T., Kashina, A.S., Davydov, I.V., Hu, R.G., An, J.Y., Seo, J.W., Du, F., Varshavsky, A., 2002. An essential role of N-terminal arginylation in cardiovascular development. Science 297, 96-99.

Le Guludec, D., Cohen-Solal, A., Delforge, J., Delahaye, N., Syrota, A., Merlet, P., 1997. Increased myocardial muscarinic receptor density in idiopathic dilated cardiomyopathy: an in vivo PET study. Circulation 96, 3416-3422.

Le Stunff, H., Milstien, S., Spiegel, S., 2004. Generation and metabolism of bioactive sphingosine-1-phosphate. J Cell Biochem 92, 882-899.

Lee, M.J., Tasaki, T., Moroi, K., An, J.Y., Kimura, S., Davydov, I.V., Kwon, Y.T., 2005. RGS4 and RGS5 are in vivo substrates of the N-end rule pathway. Proc Natl Acad Sci U S A 102, 15030-15035.

Lee, N.H., Fraser, C.M., 1993. Cross-talk between m1 muscarinic acetylcholine and beta 2-adrenergic receptors. cAMP and the third intracellular loop of m1 muscarinic receptors confer heterologous regulation. J Biol Chem 268, 7949-7957.

Li, J., Adams, L.D., Wang, X., Pabon, L., Schwartz, S.M., Sane, D.C., Geary, R.L., 2004. Regulator of G protein signaling 5 marks peripheral arterial smooth muscle cells and is downregulated in atherosclerotic plaque. J Vasc Surg 40, 519-528.

Li, Y., Hashim, S., Anand-Srivastava, M.B., 2005. Angiotensin II-evoked enhanced expression of RGS2 attenuates Gi-mediated adenylyl cyclase signaling in A10 cells. Cardiovasc Res 66, 503-511.

Liang, Y., Li, C., Guzman, V.M., Chang, W.W., Evinger, A.J., 3rd, Sao, D., Woodward, D.F., 2005. Identification of a novel alternative splicing variant of RGS5 mRNA in human ocular tissues. Febs J 272, 791-799.

Lin, F., Wang, H., Malbon, C.C., 2000. Gravin-mediated formation of signaling complexes in beta 2-adrenergic receptor desensitization and resensitization. J Biol Chem 275, 19025-19034.

Lin, F.T., Krueger, K.M., Kendall, H.E., Daaka, Y., Fredericks, Z.L., Pitcher, J.A., Lefkowitz, R.J., 1997. Clathrin-mediated endocytosis of the beta-adrenergic receptor is regulated by phosphorylation/dephosphorylation of beta-arrestin1. J Biol Chem 272, 31051-31057.

Liu, W., Yuen, E.Y., Allen, P.B., Feng, J., Greengard, P., Yan, Z., 2006. Adrenergic modulation of NMDA receptors in prefrontal cortex is differentially regulated by RGS proteins and spinophilin. Proc Natl Acad Sci U S A 103, 18338-18343.

MacEwan, D.J., Milligan, G., 1996. Inverse agonist-induced up-regulation of the human beta2-adrenoceptor in transfected neuroblastoma X glioma hybrid cells. Mol Pharmacol 50, 1479-1486.

Maguire, J.J., Davenport, A.P., 2005. Regulation of vascular reactivity by established and emerging GPCRs. Trends Pharmacol Sci 26, 448-454.

Marchese, A., Paing, M.M., Temple, B.R., Trejo, J., 2008. G protein-coupled receptor sorting to endosomes and lysosomes. Annu Rev Pharmacol Toxicol 48, 601-629.

Marianayagam, N.J., Sunde, M., Matthews, J.M., 2004. The power of two: protein dimerization in biology. Trends Biochem Sci 29, 618-625.

Martin, N.P., Lefkowitz, R.J., Shenoy, S.K., 2003. Regulation of V2 vasopressin receptor degradation by agonist-promoted ubiquitination. J Biol Chem 278, 45954-45959.

Maudsley, S., Martin, B., Luttrell, L.M., 2005. The origins of diversity and specificity in g protein-coupled receptor signaling. J Pharmacol Exp Ther 314, 485-494.

Melliti, K., Meza, U., Adams, B.A., 2001. RGS2 blocks slow muscarinic inhibition of N-type Ca(2+) channels reconstituted in a human cell line. J Physiol 532, 337-347.

Menard, L., Ferguson, S.S., Zhang, J., Lin, F.T., Lefkowitz, R.J., Caron, M.G., Barak, L.S., 1997. Synergistic regulation of beta2-adrenergic receptor sequestration: intracellular complement of beta-adrenergic receptor kinase and beta-arrestin determine kinetics of internalization. Mol Pharmacol 51, 800-808.

Metaye, T., Gibelin, H., Perdrisot, R., Kraimps, J.L., 2005. Pathophysiological roles of G-protein-coupled receptor kinases. Cell Signal 17, 917-928.

Michal, P., El-Fakahany, E.E., Dolezal, V., 2007. Muscarinic M2 receptors directly activate Gq/11 and Gs G-proteins. J Pharmacol Exp Ther 320, 607-614.

Michel, M.C., Alewijnse, A.E., 2007. Ligand-directed signaling: 50 ways to find a lover. Mol Pharmacol 72, 1097-1099.

Milligan, G., 2003. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. Mol Pharmacol 64, 1271-1276.

Milligan, G., 2004. G protein-coupled receptor dimerization: function and ligand pharmacology. Mol Pharmacol 66, 1-7.

Milligan, G., Kostenis, E., 2006. Heterotrimeric G-proteins: a short history. Br J Pharmacol 147 Suppl 1, S46-55.

Mitchell, T.S., Bradley, J., Robinson, G.S., Shima, D.T., Ng, Y.S., 2008. RGS5 expression is a quantitative measure of pericyte coverage of blood vessels. Angiogenesis 11, 141-151.

Mittmann, C., Chung, C.H., Hoppner, G., Michalek, C., Nose, M., Schuler, C., Schuh, A., Eschenhagen, T., Weil, J., Pieske, B., Hirt, S., Wieland, T., 2002. Expression of ten RGS proteins in human myocardium: functional characterization of an upregulation of RGS4 in heart failure. Cardiovasc Res 55, 778-786.

Mittmann, C., Schuler, C., Chung, C.H., Hoppner, G., Nose, M., Kehrl, J.H., Wieland, T., 2001. Evidence for a short form of RGS3 preferentially expressed in the human heart. Naunyn Schmiedebergs Arch Pharmacol 363, 456-463.

Miyoshi, K., Kawakami, N., Horio, S., Fukui, H., 2004. Homologous and heterologous phosphorylations of human histamine H1 receptor in intact cells. J Pharmacol Sci 96, 474-482.

Moffett, S., Mouillac, B., Bonin, H., Bouvier, M., 1993. Altered phosphorylation and desensitization patterns of a human beta 2-adrenergic receptor lacking the palmitoylated Cys341. Embo J 12, 349-356.

Moller, S., Vilo, J., Croning, M.D., 2001. Prediction of the coupling specificity of G protein coupled receptors to their G proteins. Bioinformatics 17 Suppl 1, S174-181.

Moniotte, S., Kobzik, L., Feron, O., Trochu, J.N., Gauthier, C., Balligand, J.L., 2001. Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. Circulation 103, 1649-1655.

Moore, C.A., Milano, S.K., Benovic, J.L., 2007. Regulation of receptor trafficking by GRKs and arrestins. Annu Rev Physiol 69, 451-482.

Moroi, K., Nishiyama, M., Kawabata, S., Ichiba, H., Yajima, T., Kimura, S., 2007. Phosphorylation of Ser166 in RGS5 by protein kinase C causes loss of RGS function. Life Sci 81, 40-50.

Mulders, A.C., Hendriks-Balk, M.C., Mathy, M.J., Michel, M.C., Alewijnse, A.E., Peters, S.L., 2006. Sphingosine kinase-dependent activation of endothelial nitric oxide synthase by angiotensin II. Arterioscler Thromb Vasc Biol 26, 2043-2048.

Mundell, S.J., Pula, G., Carswell, K., Roberts, P.J., Kelly, E., 2003. Agonist-induced internalization of metabotropic glutamate receptor 1A: structural determinants for protein kinase C- and G protein-coupled receptor kinase-mediated internalization. J Neurochem 84, 294-304.

Mundell, S.J., Pula, G., McIlhinney, R.A., Roberts, P.J., Kelly, E., 2004. Desensitization and internalization of metabotropic glutamate receptor 1a following activation of heterologous Gq/11-coupled receptors. Biochemistry 43, 7541-7551.

Ng, G.Y., Clark, J., Coulombe, N., Ethier, N., Hebert, T.E., Sullivan, R., Kargman, S., Chateauneuf, A., Tsukamoto, N., McDonald, T., Whiting, P., Mezey, E., Johnson, M.P., Liu, Q., Kolakowski, L.F., Jr., Evans, J.F., Bonner, T.I., O'Neill, G.P., 1999. Identification of a GABAB receptor subunit, gb2, required for functional GABAB receptor activity. J Biol Chem 274, 7607-7610.

Novi, F., Scarselli, M., Corsini, G.U., Maggio, R., 2004. The paired activation of the two components of the muscarinic M3 receptor dimer is required for induction of ERK1/2 phosphorylation. J Biol Chem 279, 7476-7486.

Oo, M.L., Thangada, S., Wu, M.T., Liu, C.H., Macdonald, T.L., Lynch, K.R., Lin, C.Y., Hla, T., 2007. Immunosuppressive and anti-angiogenic sphingosine 1-phosphate receptor-1 agonists induce ubiquitinylation and proteasomal degradation of the receptor. J Biol Chem 282, 9082-9089.

Osei-Owusu, P., Sun, X., Drenan, R.M., Steinberg, T.H., Blumer, K.J., 2007. Regulation of RGS2 and second messenger signaling in vascular smooth muscle cells by cGMP-dependent protein kinase. J Biol Chem 282, 31656-31665.

Oshita, A., Iwai, M., Chen, R., Ide, A., Okumura, M., Fukunaga, S., Yoshii, T., Mogi, M., Higaki, J., Horiuchi, M., 2006. Attenuation of inflammatory vascular remodeling by angiotensin II type 1 receptor-associated protein. Hypertension 48, 671-676.

Osterhout, J.L., Waheed, A.A., Hiol, A., Ward, R.J., Davey, P.C., Nini, L., Wang, J., Milligan, G., Jones, T.L., Druey, K.M., 2003. Palmitoylation regulates regulator of G-protein signaling (RGS) 16 function. II. Palmitoylation of a cysteine residue in the RGS box is critical for RGS16 GTPase accelerating activity and regulation of Gi-coupled signalling. J Biol Chem 278, 19309-19316.

Ostrom, R.S., Insel, P.A., 2004. The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology. Br J Pharmacol 143, 235-245.

Ouyang, Y.S., Tu, Y., Barker, S.A., Yang, F., 2003. Regulators of G-protein signaling (RGS) 4, insertion into model membranes and inhibition of activity by phosphatidic acid. J Biol Chem 278, 11115-11122.

Owen, V.J., Burton, P.B., Mullen, A.J., Birks, E.J., Barton, P., Yacoub, M.H., 2001. Expression of RGS3, RGS4 and Gi alpha 2 in acutely failing donor hearts and end-stage heart failure. Eur Heart J 22, 1015-1020.

Paing, M.M., Stutts, A.B., Kohout, T.A., Lefkowitz, R.J., Trejo, J., 2002. beta -Arrestins regulate protease-activated receptor-1 desensitization but not internalization or Down-regulation. J Biol Chem 277, 1292-1300.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 289, 739-745.

Panetta, R., Guo, Y., Magder, S., Greenwood, M.T., 1999. Regulators of G-protein signaling (RGS) 1 and 16 are induced in response to bacterial lipopolysaccharide and stimulate c-fos promoter expression. Biochem Biophys Res Commun 259, 550-556.

Patten, M., Bunemann, J., Thoma, B., Kramer, E., Thoenes, M., Stube, S., Mittmann, C., Wieland, T., 2002. Endotoxin induces desensitization of cardiac endothelin-1 receptor signaling by increased expression of RGS4 and RGS16. Cardiovasc Res 53, 156-164.

Penela, P., Murga, C., Ribas, C., Tutor, A.S., Peregrin, S., Mayor, F., Jr., 2006. Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease. Cardiovasc Res 69, 46-56.

Pierce, K.L., Premont, R.T., Lefkowitz, R.J., 2002. Seven-transmembrane receptors. Nat Rev Mol Cell Biol 3, 639-650.

Pitcher, J.A., Fredericks, Z.L., Stone, W.C., Premont, R.T., Stoffel, R.H., Koch, W.J., Lefkowitz, R.J., 1996. Phosphatidylinositol 4,5-bisphosphate (PIP2)-enhanced G protein-coupled receptor kinase (GRK) activity. Location, structure, and regulation of the PIP2 binding site distinguishes the GRK subfamilies. J Biol Chem 271, 24907-24913.

Pitcher, J.A., Freedman, N.J., Lefkowitz, R.J., 1998. G protein-coupled receptor kinases. Annu Rev Biochem 67, 653-692.

Pommier, B., Da Nascimento, S., Dumont, S., Bellier, B., Million, E., Garbay, C., Roques, B.P., Noble, F., 1999. The cholecystokininB receptor is coupled to two effector pathways through pertussis toxin-sensitive and -insensitive G proteins. J Neurochem 73, 281-288.

Popov, S.G., Krishna, U.M., Falck, J.R., Wilkie, T.M., 2000. Ca2+/Calmodulin reverses phosphatidylinositol 3,4, 5-trisphosphate-dependent inhibition of regulators of G protein-signaling GTPase-activating protein activity. J Biol Chem 275, 18962-18968.

Premont, R.T., Gainetdinov, R.R., 2007. Physiological roles of G protein-coupled receptor kinases and arrestins. Annu Rev Physiol 69, 511-534.

Prezeau, L., Richman, J.G., Edwards, S.W., Limbird, L.E., 1999. The zeta isoform of 14-3-3 proteins interacts with the third intracellular loop of different alpha2-adrenergic receptor subtypes. J Biol Chem 274, 13462-13469.

Prinster, S.C., Hague, C., Hall, R.A., 2005. Heterodimerization of g protein-coupled receptors: specificity and functional significance. Pharmacol Rev 57, 289-298.

Pyne, S., Pyne, N.J., 2002. Sphingosine 1-phosphate signalling and termination at lipid phosphate receptors. Biochim Biophys Acta 1582, 121-131.

Rajagopal, K., Whalen, E.J., Violin, J.D., Stiber, J.A., Rosenberg, P.B., Premont, R.T., Coffman, T.M., Rockman, H.A., Lefkowitz, R.J., 2006. Beta-arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. Proc Natl Acad Sci U S A 103, 16284-16289.

Rapacciuolo, A., Suvarna, S., Barki-Harrington, L., Luttrell, L.M., Cong, M., Lefkowitz, R.J., Rockman, H.A., 2003. Protein kinase A and G protein-coupled receptor kinase phosphorylation mediates beta-1 adrenergic receptor endocytosis through different pathways. J Biol Chem 278, 35403-35411.

Rasmussen, S.G., Choi, H.J., Rosenbaum, D.M., Kobilka, T.S., Thian, F.S., Edwards, P.C., Burghammer, M., Ratnala, V.R., Sanishvili, R., Fischetti, R.F., Schertler, G.F., Weis, W.I., Kobilka, B.K., 2007. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 450, 383-387.

Ribas, C., Penela, P., Murga, C., Salcedo, A., Garcia-Hoz, C., Jurado-Pueyo, M., Aymerich, I., Mayor, F., Jr., 2007. The G protein-coupled receptor kinase (GRK) interactome: role of GRKs in GPCR regulation and signaling. Biochim Biophys Acta 1768, 913-922.

Richman, J.G., Brady, A.E., Wang, Q., Hensel, J.L., Colbran, R.J., Limbird, L.E., 2001. Agonist-regulated Interaction between alpha2-adrenergic receptors and spinophilin. J Biol Chem 276, 15003-15008.

Riddle, E.L., Schwartzman, R.A., Bond, M., Insel, P.A., 2005. Multi-tasking RGS proteins in the heart: the next therapeutic target? Circ Res 96, 401-411.

Rogers, J.H., Tamirisa, P., Kovacs, A., Weinheimer, C., Courtois, M., Blumer, K.J., Kelly, D.P., Muslin, A.J., 1999. RGS4 causes increased mortality and reduced cardiac hypertrophy in response to pressure overload. J Clin Invest 104, 567-576.

Rose, J.J., Taylor, J.B., Shi, J., Cockett, M.I., Jones, P.G., Hepler, J.R., 2000. RGS7 is palmitoylated and exists as biochemically distinct forms. J Neurochem 75, 2103-2112.

Ross, E.M., Wilkie, T.M., 2000. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem 69, 795-827.

Roy, A.A., Baragli, A., Bernstein, L.S., Hepler, J.R., Hebert, T.E., Chidiac, P., 2006. RGS2 interacts with Gs and adenylyl cyclase in living cells. Cell Signal 18, 336-348.

Roy, A.A., Lemberg, K.E., Chidiac, P., 2003. Recruitment of RGS2 and RGS4 to the plasma membrane by G proteins and receptors reflects functional interactions. Mol Pharmacol 64, 587-593.

Rutherford, C., Ord-Shrimpton, F.U., Sands, W.A., Pediani, J.D., Benovic, J.L., McGrath, J.C., Palmer, T.M., 2005. Phosphorylation-independent internalisation and desensitisation of the human sphingosine-1-phosphate receptor S1P3. Cell Signal 17, 997-1009.

Salahpour, A., Angers, S., Mercier, J.F., Lagace, M., Marullo, S., Bouvier, M., 2004. Homodimerization of the beta2-adrenergic receptor as a prerequisite for cell surface targeting. J Biol Chem 279, 33390-33397.

Salim, S., Sinnarajah, S., Kehrl, J.H., Dessauer, C.W., 2003. Identification of RGS2 and type V adenylyl cyclase interaction sites. J Biol Chem 278, 15842-15849.

Sato, M., Horinouchi, T., Hutchinson, D.S., Evans, B.A., Summers, R.J., 2007. Ligand-directed signaling at the beta3-adrenoceptor produced by 3-(2-Ethylphenoxy)-1-[(1,S)-1,2,3,4-tetrahydronapth-1-ylamino]-2S-2-propan ol oxalate (SR59230A) relative to receptor agonists. Mol Pharmacol 72, 1359-1368.

Scheschonka, A., Dessauer, C.W., Sinnarajah, S., Chidiac, P., Shi, C.S., Kehrl, J.H., 2000. RGS3 is a GTPase-activating protein for g(ialpha) and g(qalpha) and a potent inhibitor of signaling by GTPase-deficient forms of g(qalpha) and g(11alpha). Mol Pharmacol 58, 719-728.

Schmidt, M., Sand, C., Jakobs, K.H., Michel, M.C., Weernink, P.A., 2007. Epac and the cardiovascular system. Curr Opin Pharmacol 7, 193-200.

Schnabel, P., Bohm, M., 1996. Heterotrimeric G proteins in heart disease. Cell Signal 8, 413-423.

Schoeber, J.P., Topala, C.N., Wang, X., Diepens, R.J., Lambers, T.T., Hoenderop, J.G., Bindels, R.J., 2006. RGS2 inhibits the epithelial Ca2+ channel TRPV6. J Biol Chem 281, 29669-29674.

Schwendt, M., Gold, S.J., McGinty, J.F., 2006. Acute amphetamine down-regulates RGS4 mRNA and protein expression in rat forebrain: distinct roles of D1 and D2 dopamine receptors. J Neurochem 96, 1606-1615.

Schwindinger, W.F., Robishaw, J.D., 2001. Heterotrimeric G-protein betagamma-dimers in growth and differentiation. Oncogene 20, 1653-1660.

Semplicini, A., Lenzini, L., Sartori, M., Papparella, I., Calo, L.A., Pagnin, E., Strapazzon, G., Benna, C., Costa, R., Avogaro, A., Ceolotto, G., Pessina, A.C., 2006. Reduced expression of regulator of G-protein signaling 2 (RGS2) in hypertensive patients increases calcium mobilization and ERK1/2 phosphorylation induced by angiotensin II. J Hypertens 24, 1115-1124.

Shenoy, S.K., Lefkowitz, R.J., 2003a. Multifaceted roles of beta-arrestins in the regulation of seven-membrane-spanning receptor trafficking and signalling. Biochem J 375, 503-515.

Shenoy, S.K., Lefkowitz, R.J., 2003b. Trafficking patterns of beta-arrestin and G protein-coupled receptors determined by the kinetics of beta-arrestin deubiquitination. J Biol Chem 278, 14498-14506.

Shenoy, S.K., Lefkowitz, R.J., 2005. Receptor-specific ubiquitination of beta-arrestin directs assembly and targeting of seven-transmembrane receptor signalosomes. J Biol Chem 280, 15315-15324.

Shenoy, S.K., McDonald, P.H., Kohout, T.A., Lefkowitz, R.J., 2001. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. Science 294, 1307-1313.

Shi, C.S., Lee, S.B., Sinnarajah, S., Dessauer, C.W., Rhee, S.G., Kehrl, J.H., 2001. Regulator of G-protein signaling 3 (RGS3) inhibits Gbeta1gamma 2-induced inositol phosphate production, mitogen-activated protein kinase activation, and Akt activation. J Biol Chem 276, 24293-24300.

Siderovski, D.P., Willard, F.S., 2005. The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci 1, 51-66.

Siehler, S., Manning, D.R., 2002. Pathways of transduction engaged by sphingosine 1-phosphate through G protein-coupled receptors. Biochim Biophys Acta 1582, 94-99.

Sinnarajah, S., Dessauer, C.W., Srikumar, D., Chen, J., Yuen, J., Yilma, S., Dennis, J.C., Morrison, E.E., Vodyanoy, V., Kehrl, J.H., 2001. RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III. Nature 409, 1051-1055.

Smit, M.J., Vischer, H.F., Bakker, R.A., Jongejan, A., Timmerman, H., Pardo, L., Leurs, R., 2007. Pharmacogenomic and structural analysis of constitutive g protein-coupled receptor activity. Annu Rev Pharmacol Toxicol 47, 53-87.

Smith, F.D., Oxford, G.S., Milgram, S.L., 1999. Association of the D2 dopamine receptor third cytoplasmic loop with spinophilin, a protein phosphatase-1-interacting protein. J Biol Chem 274, 19894-19900.

Snabaitis, A.K., Muntendorf, A., Wieland, T., Avkiran, M., 2005. Regulation of the extracellular signal-regulated kinase pathway in adult myocardium: differential roles of G(q/11), Gi and G(12/13) proteins in signalling by alpha1-adrenergic, endothelin-1 and thrombin-sensitive protease-activated receptors. Cell Signal 17, 655-664.

Snow, B.E., Hall, R.A., Krumins, A.M., Brothers, G.M., Bouchard, D., Brothers, C.A., Chung, S., Mangion, J., Gilman, A.G., Lefkowitz, R.J., Siderovski, D.P., 1998. GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. J Biol Chem 273, 17749-17755.

Sobue, K., Hayashi, K., Nishida, W., 1999. Expressional regulation of smooth muscle cell-specific genes in association with phenotypic modulation. Mol Cell Biochem 190, 105-118.

Stoffel, R.H., Randall, R.R., Premont, R.T., Lefkowitz, R.J., Inglese, J., 1994. Palmitoylation of G protein-coupled receptor kinase, GRK6. Lipid modification diversity in the GRK family. J Biol Chem 269, 27791-27794.

Sun, X., Kaltenbronn, K.M., Steinberg, T.H., Blumer, K.J., 2005. RGS2 is a mediator of nitric oxide action on blood pressure and vasoconstrictor signaling. Mol Pharmacol 67, 631-639.

Takeishi, Y., Jalili, T., Hoit, B.D., Kirkpatrick, D.L., Wagoner, L.E., Abraham, W.T., Walsh, R.A., 2000. Alterations in Ca2+ cycling proteins and G alpha q signaling after left ventricular assist device support in failing human hearts. Cardiovasc Res 45, 883-888.

Tanaka, Y., Tamura, K., Koide, Y., Sakai, M., Tsurumi, Y., Noda, Y., Umemura, M., Ishigami, T., Uchino, K., Kimura, K., Horiuchi, M., Umemura, S., 2005. The novel angiotensin II type 1 receptor (AT1R)-associated protein ATRAP downregulates AT1R and ameliorates cardiomyocyte hypertrophy. FEBS Lett 579, 1579-1586.

Tang, C.M., Insel, P.A., 2004. GPCR expression in the heart; "new" receptors in myocytes and fibroblasts. Trends Cardiovasc Med 14, 94-99.

Tang, K.M., Wang, G.R., Lu, P., Karas, R.H., Aronovitz, M., Heximer, S.P., Kaltenbronn, K.M., Blumer, K.J., Siderovski, D.P., Zhu, Y., Mendelsohn, M.E., 2003. Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure. Nat Med 9, 1506-1512.

Taymans, J.M., Kia, H.K., Claes, R., Cruz, C., Leysen, J., Langlois, X., 2004. Dopamine receptor-mediated regulation of RGS2 and RGS4 mRNA differentially depends on ascending dopamine projections and time. Eur J Neurosci 19, 2249-2260.

Tobin, A.B., 1997. Phosphorylation of phospholipase C-coupled receptors. Pharmacol Ther 75, 135-151.

Tovey, S.C., Willars, G.B., 2004. Single-cell imaging of intracellular Ca2+ and phospholipase C activity reveals that RGS 2, 3, and 4 differentially regulate signaling via the Galphaq/11-linked muscarinic M3 receptor. Mol Pharmacol 66, 1453-1464.

Turner, J.H., Raymond, J.R., 2005. Interaction of calmodulin with the serotonin 5-hydroxytryptamine2A receptor. A putative regulator of G protein coupling and receptor phosphorylation by protein kinase C. J Biol Chem 280, 30741-30750.

Uberti, M.A., Hague, C., Oller, H., Minneman, K.P., Hall, R.A., 2005. Heterodimerization with beta2-adrenergic receptors promotes surface expression and functional activity of alpha1D-adrenergic receptors. J Pharmacol Exp Ther 313, 16-23.

Urban, J.D., Clarke, W.P., von Zastrow, M., Nichols, D.E., Kobilka, B., Weinstein, H., Javitch, J.A., Roth, B.L., Christopoulos, A., Sexton, P.M., Miller, K.J., Spedding, M., Mailman, R.B., 2007. Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320, 1-13.

Vinge, L.E., Oie, E., Andersson, Y., Grogaard, H.K., Andersen, G., Attramadal, H., 2001. Myocardial distribution and regulation of GRK and beta-arrestin isoforms in congestive heart failure in rats. Am J Physiol Heart Circ Physiol 281, H2490-2499.

Wang, L., Mukhopadhyay, D., Xu, X., 2006. C terminus of RGS-GAIP-interacting protein conveys neuropilin-1-mediated signaling during angiogenesis. Faseb J 20, 1513-1515.

Wang, Q., Liu, M., Mullah, B., Siderovski, D.P., Neubig, R.R., 2002. Receptor-selective effects of endogenous RGS3 and RGS5 to regulate mitogen-activated protein kinase activation in rat vascular smooth muscle cells. J Biol Chem 277, 24949-24958.

Wang, X., Zeng, W., Kim, M.S., Allen, P.B., Greengard, P., Muallem, S., 2007. Spinophilin/neurabin reciprocally regulate signaling intensity by G protein-coupled receptors. Embo J 26, 2768-2776.

Wang, X., Zeng, W., Soyombo, A.A., Tang, W., Ross, E.M., Barnes, A.P., Milgram, S.L., Penninger, J.M., Allen, P.B., Greengard, P., Muallem, S., 2005. Spinophilin regulates Ca2+ signalling by binding the N-terminal domain of RGS2 and the third intracellular loop of G-protein-coupled receptors. Nat Cell Biol 7, 405-411.

Wess, J., 1997. G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. Faseb J 11, 346-354.

Wess, J., 1998. Molecular basis of receptor/G-protein-coupling selectivity. Pharmacol Ther 80, 231-264.

Wieland, T., Mittmann, C., 2003. Regulators of G-protein signalling: multifunctional proteins with impact on signalling in the cardiovascular system. Pharmacol Ther 97, 95-115.

Xie, G.X., Palmer, P.P., 2007. How regulators of G protein signaling achieve selective regulation. J Mol Biol 366, 349-365.

Xu, J., He, J., Castleberry, A.M., Balasubramanian, S., Lau, A.G., Hall, R.A., 2003. Heterodimerization of alpha 2A- and beta 1-adrenergic receptors. J Biol Chem 278, 10770-10777.

Xu, X., Zeng, W., Popov, S., Berman, D.M., Davignon, I., Yu, K., Yowe, D., Offermanns, S., Muallem, S., Wilkie, T.M., 1999. RGS proteins determine signaling specificity of Gq-coupled receptors. J Biol Chem 274, 3549-3556.

Yang, J., Kamide, K., Kokubo, Y., Takiuchi, S., Tanaka, C., Banno, M., Miwa, Y., Yoshii, M., Horio, T., Okayama, A., Tomoike, H., Kawano, Y., Miyata, T., 2005. Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. J Hypertens 23, 1497-1505.

Ying, Z., Jin, L., Dorrance, A.M., Webb, R.C., 2004. Increaseed expression of mRNA for regulator of G protein signaling domain-containing Rho guanine nucleotide exchange factors in aorta from stroke-prone spontaneously hypertensive rats. Am J Hypertens 17, 981-985.

Yoburn, B.C., Purohit, V., Patel, K., Zhang, Q., 2004. Opioid agonist and antagonist treatment differentially regulates immunoreactive mu-opioid receptors and dynamin-2 in vivo. Eur J Pharmacol 498, 87-96.

Zaugg, M., Schaub, M.C., 2004. Cellular mechanisms in sympatho-modulation of the heart. Br J Anaesth 93, 34-52.

Zeng, W., Xu, X., Popov, S., Mukhopadhyay, S., Chidiac, P., Swistok, J., Danho, W., Yagaloff, K.A., Fisher, S.L., Ross, E.M., Muallem, S., Wilkie, T.M., 1998. The N-terminal domain of RGS4 confers receptor-selective inhibition of G protein signaling. J Biol Chem 273, 34687-34690.

Zhang, W., Anger, T., Su, J., Hao, J., Xu, X., Zhu, M., Gach, A., Cui, L., Liao, R., Mende, U., 2006. Selective loss of fine tuning of Gq/11 signaling by RGS2 protein exacerbates cardiomyocyte hypertrophy. J Biol Chem 281, 5811-5820.

Zheng, B., Ma, Y.C., Ostrom, R.S., Lavoie, C., Gill, G.N., Insel, P.A., Huang, X.Y., Farquhar, M.G., 2001. RGS-PX1, a GAP for GalphaS and sorting nexin in vesicular trafficking. Science 294, 1939-1942.

Zhong, H., Wade, S.M., Woolf, P.J., Linderman, J.J., Traynor, J.R., Neubig, R.R., 2003. A spatial focusing model for G protein signals. Regulator of G protein signaling (RGS) protien-mediated kinetic scaffolding. J Biol Chem 278, 7278-7284.

Zhou, J., Moroi, K., Nishiyama, M., Usui, H., Seki, N., Ishida, J., Fukamizu, A., Kimura, S., 2001. Characterization of RGS5 in regulation of G protein-coupled receptor signaling. Life Sci 68, 1457-1469.

Zhu, W.Z., Chakir, K., Zhang, S., Yang, D., Lavoie, C., Bouvier, M., Hebert, T.E., Lakatta, E.G., Cheng, H., Xiao, R.P., 2005. Heterodimerization of beta1- and beta2-adrenergic receptor subtypes optimizes beta-adrenergic modulation of cardiac contractility. Circ Res 97, 244-251.

Zou, M.X., Roy, A.A., Zhao, Q., Kirshenbaum, L.A., Karmazyn, M., Chidiac, P., 2006. RGS2 is upregulated by and attenuates the hypertrophic effect of alpha1-adrenergic activation in cultured ventricular myocytes. Cell Signal 18, 1655-1663.