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

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Chapter 1

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 \( \alpha \)-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.
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 β₂-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 βγ-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

![Image of Fig. 1](image-url)  
**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.
proteins are divided into four families: $G_s$, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$ based on similarity of $\alpha$-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 $G\alpha$ subunit (Hermans, 2003), such as the $M_2$ muscarinic receptor which can couple to $G_s$, $G_i$, 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 $\alpha$-subunit, thereby generating active GTP-bound $G\alpha$ subunit from the inactive GDP-bound state followed by dissociation from the receptor and $G\beta\gamma$ subunit. Both the $G\alpha$ and $G\beta\gamma$ subunit can modulate the activity of downstream effectors. Specific $G\alpha$ 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 $G\beta\gamma$ 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), PLC$\beta$, AC and the small GTP binding proteins (Cabrera-Vera et al., 2003; Schwindinger and Robishaw,
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 Gq/11 Signalling of GPCRs, PLC activation and subsequent IP₃ and diacylglycerol formation (Allen and Walsh, 1994). Activation of IP₃ 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 G10⁻ mediated activation of PLC and inhibition of AC or G₁₂/₁₃ – 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 AT₁ receptors, endothelin-1 ET₄ receptors and α₁-adrenoceptors mainly use the Gq/11-PLC signalling pathway to stimulate vasoconstriction whereas other receptors, such as the α₂-adrenoceptors, mediate contraction via G10 proteins (Maguire and Davenport, 2005).

Relaxation of vascular smooth muscle cells is mainly mediated by Gi protein-coupled receptors, which reduce the Ca²⁺ sensitivity. In addition, Gq/11 and G₁₀ 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 Ca²⁺ sensitivity via Gi 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 M₁ and M₃ 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). $G_{o/k}$ 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 $\beta_1$-adrenoceptor by sympathetic activity and to a lesser extent via the $\beta_2$-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 $G_{o/k}$-mediated inhibition of AC and inhibition of HCN channel activity, and by $G_{i/o}$-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 $\beta_2$-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 $\alpha_1$-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 β2-adrenoceptor is involved in the attenuation of its inotropic effects and causes a shift in G protein-coupling selectivity towards Gi proteins (Daaka et al., 1997a). This shift from Gi towards Gs protein signalling further attenuates the positive inotropic effects of the β2-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 Gβγ 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 α1B-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 S1P3 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 phosphorylation 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 ETB 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 β2-adrenoceptor, whereas other receptors recycle more slowly, such as the angiotensin II AT1a and vasopressin V2 receptor (Shenoy and Lefkowitz, 2003b). This difference in recycling rate is caused by the difference in stability of the GPCR-arrestin complex. The β2-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 AT1a and vasopressin V2 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.
Regulation of GPCR signalling. A general introduction

Chapter 1

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 (Brode, 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.
Chapter 1

**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_{i/o}, 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$_R_1$ receptor, which requires both subunits to be a fully functional receptor. The GABA$_R_1$ and GABA$_R_2$ receptors are non-functional as monomers. Only GABA$_R_1$-R$_2$ 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 $\alpha_{1D}$-adrenoceptor can form a heterodimer with the $\alpha_{1B}$- and the $\beta_2$-adrenoceptor, but not with the $\alpha_{1A}$-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 α₁, β₁- and β₂-adrenoceptors, angiotensin II AT₁ receptor, adenosine A₁ receptor and the M₂ 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 β₂-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 β₂-adrenoceptor, PKA anchored to AKAP79, is activated and phosphorylates the third intracellular loop of the receptor. This phosphorylation causes the receptor to switch from Gs coupling to Gi coupling and promotes MAPK signalling. By facilitation 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 β₂-adrenoceptor (Appert-Collin et al., 2006). Interaction of AKAP250 with the β₂-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 β₂-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 β₂-adrenoceptor without G protein activation (Hall et al., 1998a; Hall et al., 1998b). Direct interaction with NHERF1 also mediates recycling of internalized β₂-adrenoceptors to the membrane (Cao et al., 1999).

An important GIP for the angiotensin II AT₁ receptor is the AT₁ 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 AT₁-mediated cell proliferation and vascular remodeling by enhancement of AT₁ receptor internalization and down-regulation (Azuma et al., 2007; Oshita et al., 2006). By controlling AT₁ 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 AT1 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-Mg2+-AlF4- 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 RGSS 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

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**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>
<thead>
<tr>
<th>GPCR</th>
<th>G protein coupling</th>
<th>Effective RGS</th>
<th>Ineffective RGS</th>
<th>Functional effect</th>
<th>Cell type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II AT$_1$</td>
<td>G$_{q/11}$ (G$_i$)</td>
<td>RGS 1, RGS 2, RGS 3L, RGS 4$^1$</td>
<td>RGS 2, RGS 3, RGS 7$^2$</td>
<td>MAPK activity ↓</td>
<td>HeLa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 5$^1$</td>
<td></td>
<td>MAPK activity ↓</td>
<td>VSMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 5$^2$</td>
<td></td>
<td>IP formation ↓</td>
<td>VSMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2$^3$</td>
<td></td>
<td>Inhibition of cAMP formation ↓</td>
<td>VSMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2$^4$</td>
<td></td>
<td>IP formation ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 4, RGS 5$^5$</td>
<td></td>
<td>Ca$^{2+}$ response ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td>α$_x$-adrenoceptor</td>
<td>G$_{q/11}$</td>
<td>RGS 2$^6$</td>
<td>RGS 16$^6$</td>
<td>IP formation ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>α$_y$-adrenoceptor</td>
<td>G$_{q/11}$</td>
<td>nd</td>
<td>RGS 2$^6$</td>
<td>IP formation ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>α$_x$-adrenoceptor</td>
<td>G$_{q/11}$</td>
<td>RGS 2, RGS 4$^{14}$</td>
<td></td>
<td>MAPK activity ↓</td>
<td>ARVM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2$^{15}$</td>
<td></td>
<td>MAPK activity ↓, JNK activity</td>
<td>Cardiomyocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2$^{16}$, RGS 3, RGS 4, RGS 5$^{17}$</td>
<td></td>
<td>IP formation ↓</td>
<td>Cardiomyocytes</td>
</tr>
<tr>
<td>β$_2$-adrenoceptor</td>
<td>G$_i$ (G$_i$)</td>
<td>RGS 2$^7$</td>
<td>nd</td>
<td>cAMP formation ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>Endothelin ET$_A$</td>
<td>G$_{q/11}$ (G$_i$)</td>
<td>RGS 3L, RGS 4$^4$</td>
<td>RGS 1, RGS 2$^1$</td>
<td>MAPK activity ↓</td>
<td>HeLa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 4, RGS 5$^5$</td>
<td></td>
<td>Ca$^{2+}$ response ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td>Endothelin ET$_X$</td>
<td>G$_{q/11}$ (G$_i$)</td>
<td>RGS 2, RGS 4$^{14}$</td>
<td></td>
<td>MAPK activity ↓</td>
<td>ARVM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2$^{16}$, RGS 3, RGS 4, RGS 5$^{17}$</td>
<td></td>
<td>IP formation ↓</td>
<td>Cardiomyocytes</td>
</tr>
<tr>
<td>Muscarinic M$_1$</td>
<td>G$_{q/11}$</td>
<td>RGS 2, RGS 3, RGS 8$^4$</td>
<td>RGS 4, RGS 5$^4$</td>
<td>Ca$^{2+}$ response ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2, RGS 8$^4$</td>
<td></td>
<td>IP formation ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2, RGS 16$^6$</td>
<td></td>
<td>IP formation ↓</td>
<td>CHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS 2, RGS 5$^9$</td>
<td></td>
<td>IP formation ↓, Ca$^{2+}$ response ↓</td>
<td>COS-7, HEK293</td>
</tr>
<tr>
<td>Muscarinic M₂</td>
<td>G₁</td>
<td>RGS₁, RGS₃, RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>GIRK current</td>
<td>Oocytes, CHO</td>
</tr>
<tr>
<td>--------------</td>
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<td>----------------</td>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₃s, RGS₃, RGS₄, RGS₅, RGS₁₆&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>COS-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₃s, RGS₃, RGS₄, RGS₅, RGS₁₆&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>AKT activity ↓</td>
<td>COS-7</td>
</tr>
<tr>
<td>Muscarinic M₃</td>
<td>G₃/₁₁</td>
<td>RGS₃&lt;sup&gt;2&lt;/sup&gt;</td>
<td>RGS₂, RGS₅, RGS₇&lt;sup&gt;2&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>VSMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₅, RGS₄&lt;sup&gt;4&lt;/sup&gt;</td>
<td>RGS₄&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Ca²⁺ response ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₅, RGS₁₆&lt;sup&gt;1/₁₃&lt;/sup&gt;</td>
<td>RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>IP formation ↓</td>
<td>COS-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₅&lt;sup&gt;1/₁₂&lt;/sup&gt;</td>
<td>RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>IP formation ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₃, RGS₄₁₂</td>
<td>RGS₂, RGS₅, RGS₄₁₂</td>
<td>Ca²⁺ response ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₃s, RGS₃, RGS₅&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂, RGS₅₁₆&lt;sup&gt;1/₁₃&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>COS-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₃s, RGS₃&lt;sup&gt;1/₁&lt;/sup&gt;</td>
<td>RGS₂, RGS₄₁₆&lt;sup&gt;1&lt;/sup&gt;</td>
<td>AKT activity ↓</td>
<td>COS-7</td>
</tr>
<tr>
<td>Muscarinic M₅</td>
<td>G₃/₁₁</td>
<td>RGS₂, RGS₃, RGS₅&lt;sup&gt;4&lt;/sup&gt;</td>
<td>RGS₃&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Ca²⁺ response ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₂, RGS₅&lt;sup&gt;4&lt;/sup&gt;</td>
<td>RGS₄&lt;sup&gt;4&lt;/sup&gt;</td>
<td>IP formation ↓</td>
<td>HEK293T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RGS₁, RGS₃L, RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>Sphingosine-1-phosphate</td>
<td>G₁</td>
<td>RGS₁, RGS₃L, RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>S1P₁</td>
<td>G₁</td>
<td>RGS₁, RGS₃L, RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>Sphingosine-1-phosphate</td>
<td>G₁, G₃/₁/₁, G₁₃/₂/₁₁</td>
<td>RGS₁, RGS₃L, RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₂&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>HEK293</td>
</tr>
<tr>
<td>S1P₂</td>
<td>G₁, G₃/₁/₁, G₁₃/₂/₁₁</td>
<td>RGS₁, RGS₂, RGS₃L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>RGS₄&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MAPK activity ↓</td>
<td>HEK293</td>
</tr>
</tbody>
</table>

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. (Cho et al., 2003a), (Wang et al., 2002), (Li et al., 2005), (Bodenstein et al., 2007), (Zhou et al., 2001), (Hague et al., 2005), (Roy et al., 2006), (Bernstein et al., 2004), (Gu et al., 2007), (Doupnik et al., 1997), (Anger et al., 2004), (Tovey and Willars, 2004), (Anger et al., 2007), (Snabaitis et al., 2005), (Zou et al., 2006), (Zhang et al., 2006), (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\textsubscript{\alpha_i} and G\textsubscript{\alpha_q/11} proteins (Hollinger and Hepler, 2002). To date only RGS-PX1 has been found to show GAP activity towards G\textsubscript{\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\textsubscript{\alpha_{12/13}} proteins.

There is increasing evidence that RGS proteins are not only able to discriminate between G\textsubscript{\alpha} subfamilies but that they are also able to discriminate between GPCRs coupled to the same G\textsubscript{\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\textsubscript{\alpha_q/11} signalling of the angiotensin II AT\textsubscript{1} and the endothelin ET\textsubscript{A} receptor can be attenuated by RGS5, whereas signalling of the G\textsubscript{\alpha_q/11}-coupled muscarinic M\textsubscript{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.

<table>
<thead>
<tr>
<th>GPCR</th>
<th>Direct interacting RGS</th>
<th>No interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>α\textsubscript{1A} adrenoceptor (i3 loop)\textsuperscript{1}</td>
<td>RGS2, RGS16</td>
<td></td>
</tr>
<tr>
<td>α\textsubscript{1B} adrenoceptor (i3 loop)\textsuperscript{2}</td>
<td>RGS2, RGS16</td>
<td></td>
</tr>
<tr>
<td>α\textsubscript{1D} adrenoceptor (i3 loop)\textsuperscript{3}</td>
<td>RGS2, RGS16</td>
<td></td>
</tr>
<tr>
<td>β\textsubscript{2} adrenoceptor (i3 loop)\textsuperscript{4}</td>
<td>RGS12</td>
<td>RGS16</td>
</tr>
<tr>
<td>CXCR2 (C-term)\textsuperscript{5}</td>
<td>RGS2, RGS4, RGS8</td>
<td>RGS1, RGS16</td>
</tr>
<tr>
<td>M\textsubscript{1} muscarinic (i3 loop)\textsuperscript{6}</td>
<td>RGS2, RGS5</td>
<td>RGS1, RGS16</td>
</tr>
<tr>
<td>M\textsubscript{2} muscarinic (i3 loop)\textsuperscript{6}</td>
<td>RGS2, RGS5</td>
<td>RGS1, RGS16</td>
</tr>
<tr>
<td>M\textsubscript{3} muscarinic (i3 loop)\textsuperscript{6}</td>
<td>RGS1, RGS5</td>
<td>RGS1, RGS16</td>
</tr>
<tr>
<td>M\textsubscript{5} muscarinic (i3 loop)\textsuperscript{6}</td>
<td>RGS1, RGS5</td>
<td>RGS1, RGS16</td>
</tr>
<tr>
<td>δ-opioid (i3 loop and C-term)\textsuperscript{7}</td>
<td>RGS4</td>
<td>RGS1, RGS16</td>
</tr>
<tr>
<td>μ-opioid (C-term)\textsuperscript{7}</td>
<td>RGS4</td>
<td>RGS1, RGS16</td>
</tr>
</tbody>
</table>

\textsuperscript{1}(Hague et al., 2005), \textsuperscript{2}(Roy et al., 2006), \textsuperscript{3}(Snow et al., 1998), \textsuperscript{4}(Bernstein et al., 2004), \textsuperscript{5}(Itoh et al., 2006), \textsuperscript{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<sub>1A</sub>- but not of the α<sub>1B</sub>- or α<sub>1D</sub>-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<sub>1A</sub>-adrenoceptor with RGS2 since mutation abolished this interaction (Hague et al., 2005). In addition, mutated α<sub>1A</sub>-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<sub>1B</sub>-adrenoceptor, explaining the lack of RGS2 interaction of the α<sub>1B</sub>-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<sub>q/11</sub>-coupled α<sub>1A</sub>-adrenoceptor (Hague et al., 2005) and angiotensin II AT<sub>1</sub> receptor and G<sub>i</sub>-coupled β<sub>2</sub>-adrenoceptor (Roy et al., 2003) are able to recruit RGS2 whereas the G<sub>i</sub>-coupled muscarinic M<sub>2</sub> receptors cannot (Roy et al., 2003). In contrast, RGS4 is translocated to the membrane by M<sub>2</sub> but not by AT<sub>1</sub> 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 Gα subunit thereby preventing the Gα-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<sub>q</sub>, 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<sub>q/11</sub> 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 Gα<sub>q/11</sub>-PLCβ interaction by competitively binding to Gα<sub>q/11</sub>. However, RGS4 was shown to interact directly with both Gα<sub>q</sub> and PLCβ, suggesting a signalling complex in which RGS4 can rapidly attenuate the G<sub>q/11</sub> signalling (Dowal et al., 2000).
2001). Beside effector antagonism on $G_{\alpha}$, 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), $G_{\beta\gamma}$ (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, $G_{\alpha}$ 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 \( \text{G}_{q/11} \) is increased upon phosphorylation by activated PKG1-\( \alpha \), resulting in a decrease of \( \text{G}_{q/11} \)-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 \( \text{Ca}^{2+} / \text{calmodulin} \), which competes for the same interaction site in the RGS domain of RGS4 (Ishii et al., 2005). The reciprocal regulation by PIP\(_3\) and \( \text{Ca}^{2+} / \text{calmodulin} \) 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 \( \text{Ca}^{2+} / \text{calmodulin} \) (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 muscle 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.,...
Regulation of GPCR signalling. A general introduction

Chapter 1

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 $G_{\text{q/11}}$-mediated signalling RGS2 attenuates contraction in vascular smooth muscle cells.

Muscarinic M2 and adenosine A1 receptor-mediated negative chronotropic effects were enhanced by knock-in of RGS-insensitive $G_{\alpha_{i}}$ (Fu et al., 2006). In addition, knock-in of RGS-insensitive $G_{\alpha_{12}}$ enhanced muscarinic M2 but not adenosine A1 receptor-mediated negative chronotropic effects, whereas $\beta_2$-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 described 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\textsubscript{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\textsubscript{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\textsubscript{q/11} signalling, resulting in an increased mortality after pressure overload (Rogers et al., 1999). However, in mice overexpressing G\textsubscript{q} an increase in RGS4 expression was beneficial in cardiomyopathy (Rogers et al., 1999). This is possibly due to attenuation of G\textsubscript{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\textsubscript{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\textsubscript{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<sub>1</sub> receptor (Oo et al., 2007) but not for the S1P<sub>3</sub> receptor, a receptor involved in the cardiac side-effects of the immunosuppressant pro-drug FTY720. Since it was postulated that internalization of the S1P<sub>1</sub> 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<sub>3</sub> 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).
Regulation of GPCR signalling. A general introduction

REFERENCES


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


Regulation of GPCR signalling. A general introduction

Chapter 1


Regulation of GPCR signalling. A general introduction

Chapter 1


Regulation of GPCR signalling. A general introduction

Chapter 1


Regulation of GPCR signalling. A general introduction

Chapter 1


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-tetrahydronaph-1-ylamino]-2S-2-propanol 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(alpha) and g(qalpha) and a potent inhibitor of signaling by GTPase-deficient forms of g(qalpha) and g(11alpha). Mol Pharmacol 58, 719-728.


Regulation of GPCR signalling. A general introduction


