Introduction:

The normal pulmonary circulation is a low resistance and high flow circulation, which is maintained by locally produced or circulating vasomodulators. An imbalance in the activity of vasoconstrictor/proliferative and vasodilator/anti-proliferative mediators in the pulmonary circulation leads to remodelling of the pulmonary artery. Structural changes in the pulmonary artery, a key feature of which is the proliferation of pulmonary artery smooth muscle cells (PASMC) leads to increased pulmonary vascular tone that can manifest as pulmonary arterial hypertension (PAH). PAH is a progressive disease that is characterised by pulmonary artery pressure greater than 25 mmHg (Lau et al., 2017); right ventricular function and hypertrophy are major determinants in the prognosis of PAH (Maarman et al., 2017). PAH, includes patients with similar pathophysiological, histological and prognostic features; PAH can be idiopathic (IPAH), heritable (70% of which are associated with mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene), or secondary to drug/toxin exposure or to other conditions, such as connective tissue disease (Lau et al., 2017). Restoring the imbalance in pulmonary vascular tone is a key end point of drugs used clinically to treat PAH.

Many of the vasoactive mediators in the pulmonary circulation, such as endothelin (ET-1), angiotensin II (Ang-II), 5-hydroxytryptamine (5-HT), prostacyclin (PGI_2) and vasoactive intestinal peptide (VIP), act via G protein coupled receptors (GPCRs) expressed on the vasculature, in particular on PASMC and pulmonary artery endothelial cells (PAEC) (Barnes and Liu, 1995; Morrell *et al.*, 2009; Murray *et al.,* 2011). GPCRs are the largest receptor family in the human genome and successful therapeutic targets due to their tissue and cell specific distribution and accessibility on the plasma membrane (Insel et al., 2012). Altered expression and function of a number of GPCRs and circulating levels of their endogenous ligand are associated with the progression of PAH, which when taken together, contributes to the increased pulmonary vascular tone by tipping the balance of homeostatic signalling in PASMC to favour vasoconstriction and proliferation. We will provide an overview of GPCRs, in particular those whose expression are altered in PASMC with PAH, and discuss how both “old” and “new” GPCRs are relevant targets to restore the imbalance in pulmonary vascular tone.

GPCRs in the pulmonary circulation:

GPCRs are guanine nucleotide exchange factors for heterotrimeric G proteins, whose α and βγ subunits dissociate upon ligand binding leading to the activation/inactivation of signalling pathways that control the production of second messengers, the activity of intracellular proteins and the expression of various genes (Rajagopal and Lefkowitz, 2010; Murray et al., 2011; Insel *et al.*, 2012). Although GPCRs can couple to more than one G protein, they are usually classified based on the G protein they preferentially activate. G proteins are divided into four main classes according to their α subunit: $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$, although Gβγ can also act as a single entity to initiate signalling. G protein-independent signalling also occurs on GPCR activation via β-arrestin recruitment that contributes to GPCR internalisation and downstream signalling such as ERK activation, gene transcription and growth factor receptor transactivation (Figure 1). Both G protein and β-arrestin mediated signalling are key components in a complex signalling network that controls the pulmonary circulation, however for many GPCRs the relative contribution of each of these pathways to the overall physiological response of ligands in the pulmonary artery remains to be fully explored (Murray et al., 2011).

In general, activating $G\alpha_i$-, $G\alpha_{q/11}$- and $G\alpha_{12/13}$-dependent signalling leads to vasoconstriction and proliferation of PASMC, whereas $G\alpha_s$-dependent signalling, leads to vasodilation and decreased proliferation (Figure 1). $G\alpha_s$-coupled GPCRs increases $[cAMP]_i$, by activating adenylyl cyclases, which increases the activity of downstream mediators such as protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac). PKA also phosphorylates targets such as myosin light chain (MLC) kinase to decrease its activity, whereas Epac increase Rap-1, both resulting in vasodilation and decreased proliferation of PASMC. The ability of cAMP to also regulate gene transcription, for example via the cAMP response element binding protein (CREB), means its physiological effects can persist long after GPCR activation. In contrast, activation of $G\alpha_i$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ tend to produce overlapping biological responses in PASMC, which leads to increased Ca^{2+} sensitisation and the phosphorylation of myosin light chain (MLC), promoting actin–myosin cross-bridging and PASMC contraction. Activation of $G\alpha_i$-coupled GPCRs oppose the effects of $G\alpha_s$-coupled GPCRs by decreasing $[cAMP]_i$ by inhibiting adenylyl cyclases. $G\alpha_{q/11}$-coupled GPCRs activate phospholipase C (PLC) leading to increased inositol-1,4,5-

triphosphate (IP_3) and $[Ca^{2+}]_i$ and the phosphorylation of target proteins, such as Ca^{2+}-calmodulin dependent protein kinase that activates MLC kinase, leading to vasoconstriction. In parallel, diacylglycerol (DAG) promotes the association of protein kinase C (PKC) to the membrane, which phosphorylates a number of contractile proteins. Increased Ca^{2+} sensitisation and sustained PASMC vasoconstriction can also result in stimulation of $G\alpha_{12/13}$-coupled GPCRS, which activate Rho GEFs (a low molecular weight monomeric G protein) and Rho kinase (ROCK) that phosphorylate the MLC phosphatase and inhibits its activity; $G\alpha_{q/11}$ also increases ROCK. The expression and activity of GPCRs is an important determinant in the amplitude of second messengers and downstream signalling in PASMC.

An updated list of GPCRs that are known regulators of the pulmonary vascular circulation is provided in Table 1. In PASMC at least 33 GPCRs have been characterised, some with multiple coupling; $G\alpha_s$ (11 GPCRs), $G\alpha_i$ (8 GPCRs), $G\alpha_{q/11}$ (16 GPCRs), and $G\alpha_{12/13}$ (4 GPCRs). In parallel, in PAEC at least 18 GPCRs have been characterised, again with multiple coupling; $G\alpha_s$ (2 GPCRs), $G\alpha_i$ (5 GPCRs), $G\alpha_{q/11}$ (14 GPCRs), and $G\alpha_{12/13}$ (11 GPCRs). Taken together these data show that the low tone of the pulmonary circulation is, at least in part, the consequence of the relative high abundance of $G\alpha_q/G\alpha_{12/13}$-coupled GPCRs in PAEC and $G\alpha_s$-coupled GPCRs in PASMC. GPCR ligands can have differing effects in the pulmonary circulation depending on the expression of the predominant receptor subtype, the function of the endothelium, the species being investigated and the initial tone of the pulmonary circulation; low basal tone in the pulmonary artery means vasodilators have little effect (Barnes and Liu, 1995; Murray *et al.,* 2011). ET-1 or 5-HT antagonists do not vasodilate the normal pulmonary circulation but can attenuate hypoxia or disease induced pulmonary vasoconstriction where tone is increased (Bonvallet *et al.*, 1994; Barnes and Liu, 1995; Murray *et al.,*, 2011). An intact endothelium is vital for maintaining the low tone of the pulmonary circulation. Endothelium dysfunction, as seen in PAH, can shift the response of circulating mediators to vasoconstriction since many of the endogenous mediators that stimulate release of nitric oxide (NO) and PGI_2 via GPCRs on PAEC lead to vasoconstriction if they directly act on PASMC, via $G\alpha_i/G\alpha_{q/11}/G\alpha_{12/13}$ (Morrell et al., 2009). Once endothelial dependent relaxation is attenuated, the expression and activity of GPCRs on PASMC drive remodelling and

increased vasoconstriction of the pulmonary artery; PASMC are an important cellular target for PAH.

PAH-PASMC have decreased cAMP and increased $[Ca^{2+}]_i$ compared to control-PASMC (Zhang *et al.*, 2007; Murray *et al.,* 2011), which can be attributed at least in part, to the altered expression and/or activity of GPCRs in PASMC; circulating or tissue levels of endogenous GPCR agonists are seen in PAH (Table 2). A number of GPCR agonists and antagonists, as reviewed in Table 2, have been shown to reverse or blunt PAH both clinically and pre-clinically by restoring the balance of second messengers in PASMC; PGI_2 (IP) receptor and ET-1 receptors are the targets of drugs currently approved to treat PAH (Lau et al., 2017). GPCRs whose altered expression or activity contribute to the imbalance of pulmonary vascular tone with PAH are outlined below: a $G\alpha_i/G\alpha_q/G_{12/13}$ vs. $G\alpha_s$ shift is evident with PAH.

GPCRs that contribute to the imbalance of pulmonary vascular tone with PAH

Prostanoid receptors:

Prostanoid receptors, which include $DP_{1,2}$, $EP_{1\text{-}4}$, FP, IP and TP, are activated by prostaglandin D_2 (PGD_2), prostaglandin E_1 and E_2 (PGE_1, PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGI_2, and prostaglandin H_2 (PGH_2)/thromboxane A2 (TXA_2), respectively. IP, EP_2, EP_4 and DP_1 are $G\alpha_s$-coupled and therefore increase cAMP and are vasodilatory in PASMC, whereas EP_1, EP_3, and FP and TXA are $G\alpha_{q/11}$ or $G\alpha_i$-coupled so increase $[Ca^{2+}]_i$ or decrease cAMP and lead to vasoconstriction of PASMC (Hirata and Narumiya, 2011). The main eicosanoids, produced via metabolism of arachidonic acid, in the pulmonary circulation are PGI_2 and PGE_2, which are vasodilators, and $PGF_{2\alpha}$ and TXA_2, which are vasoconstrictors; PGI_2 synthase predominates in PAEC and directs metabolism toward PGI_2, which acts on PASMC to keep normal pulmonary tone low. This homeostatic balance is however dysregulated in PAH, which results in decreased levels of PGI_2- and increased levels of TXA_2- in lungs and urine of PAH patients (Christman et al., 1992). Increasing PGI_2 production by overexpressing PGI synthase in mice prevents the development of PAH (Geraci et al., 1999). Intravenous prostacyclin (epoprostenol) and more stable, inhaled and/or orally active prostacyclin analogues, such as treprostinil and iloprost, are approved in the UK for PAH and

improve haemodynamics and exercise tolerance, long-term survival in patients and importantly reduce the need for lung transplantation (McLaughlin et al., 2015).

All prostanoid receptors are expressed at the level of mRNA in the pulmonary circulation, however the extent to which they control vascular tone is not fully understood (Hirata and Narumiya, 2011). In the human pulmonary artery the IP receptor, a $G\alpha_s$-coupled GPCR whose activation increases cAMP, is highly expressed and functional and the primary therapeutic target of the prostacyclin analogues; the severity of hypoxic-induced PAH is greater in IP receptor-deficient mice (Hoshikawa et al., 2001; Falcetti et al., 2010). PAH is associated with reduced IP and DP receptor expression (both $G\alpha_s$-coupled) -and increased EP_3 receptor expression ($G\alpha_i$-coupled), which taken together could attenuate the vasodilatory effect of endogenous eicosanoids in PASMC (Table 2). Since each prostacyclin analogue has a different pharmacological profile, altered prostanoid receptor expression may even determine their full clinical response. For example, in addition to the IP receptor, iloprost has high affinity for EP_1 receptors whose activation in PASMC would initiate vasoconstriction, whereas treprostinil has high affinity for DP_1 and EP_2 receptors whose activation would enhance PASMC vasodilation (Whittle et al., 2012): reduced IP and DP expression could blunt the vasodilatory response of these analogues. Furthermore, prostacyclin analogues have also been shown to have prostanoid receptor independent effects, via KCNK3, clearing ET-1 and PPAR-γ (Olschewski et al., 2006; Falcetti et al., 2007). Highly selective IP receptor agonists, such as selexipag and its active metabolite MRE-269/ACT-333679 have been developed and shown to reduce PASMC proliferation, inhibit PAH in models of the disease and relax isolated pulmonary artery (Morrison et al., 2012; Fuchikami et al., 2017). Selexipag decreases the risk of morbidity/mortality of PAH patients alone, or in combination with other therapies (McLaughlin et al., 2015).

A major problem with the use of most GPCR agonists is that their biological response can diminish over time, which requires that the dose needs to be increased to maintain efficacy (Lefkowitz, 1993). Such desensitisation can be attributed to receptor phosphorylation and internalization and reduced receptor expression. Desensitisation and internalisation of the IP receptor has been seen both *in-vitro* and *in-vivo*, which attenuates the vasoreactivity of prostacyclin analogues (Schermuly et al., 2007); recent advances in the pharmacology of the IP receptor could help reduce receptor

desensitisation and enhance the efficacy of drugs. Of interest, MRE-269/ACT-333679 has been shown to act as a full agonist in terms of vasodilation and inhibition of PASMC proliferation, but a partial agonist in terms of recruitment of β-arrestin and IP receptor internalization. *In-vivo* this pharmacological profile translates to sustained efficacy in animal models of PAH due to limited IP receptor desensitisation (Morrison et al., 2012). Furthermore, an IP positive allosteric modulator (PAM) has been developed (IPPAM) (Yamamoto et al., 2017). PAMs are ligands that act at on allosteric sites to increase receptor function and potentiate the activity of the orthosteric ligand (Lefkowitz, 1993). PAMS have no intrinsic activity, increased selectivity and can also reduce receptor desensitisation, therefore could have exciting therapeutic potential for PAH. IPPAM has been shown to enhancing the effects of PGI2 *in-vitro*, however *in-vivo* pre-clinical studies in models of PAH have yet to be completed (Yamamoto et al., 2017). Understanding the mechanism of the reduced expression of IP receptor with PAH and advances in drugs design continue to enforce the benefit of targeting this receptor in PAH.

Vasoactive intestinal peptide Receptors:

Vasoactive intestinal peptide (VIP) and the related pituitary adenylate cyclase–activating polypeptide (PACAP) are potent vasodilators of the pulmonary circulation and inhibit PASMC proliferation and platelet activation (Said, 2012). VIP has shown a protective role in the presence of pulmonary vasoconstrictors such as ET-1 and attenuates or reverses the development of PAH in animal models (Boomsma et al., 1991; Hamidi, 2005; Hamidi et al., 2011). The effects of VIP and PACAP are mediated by VIP receptors (VPAC1 and VPAC2) and PAC1 receptors, which are primarily $G\alpha_s$-coupled and expressed in PASMC (Busto et al., 2000); VPAC2 is highest expressed in human PASMC. PAH is associated with increased expression of VPAC1 and 2 (Petkov *et al.*, 2003; unpublished data), which can be speculated to be a compensatory mechanism due to reduced serum VIP levels in PAH patients. One interesting observation, that could be important in relation to the increased VPAC receptor expression associated with PAH, is that VIP activates PLC and increases $[Ca^{2+}]_i$ in stable cell lines overexpressing VPAC (MacKenzie et al., 1996). It would be interesting to determine if VIP mediated G protein-dependent signalling differs in PAH-PASMC.

Mice lacking the VIP gene develop a moderate form of PAH and right ventricular hypertrophy, which is attenuated by VIP treatment (Said et al., 2007). PAC1 KO mice develop PAH soon after birth, which suggest this receptor may also be key in the regulation of pulmonary vascular tone (Otto, 2004). However, VPAC2 KO mice do not develop PAH, and pulmonary remodelling has not been reported in VPAC1 KO mice, suggesting these receptors may have redundant roles in PASMC (Asnicar et al., 2002; Fabricius et al., 2011). VPAC2, but not VPAC1 selective agonists have been shown to improve right ventricular systolic pressure, in animal models of PAH, implying that VPAC2 could be a more promising target for PAH (Koga et al., 2014). Although original clinical trials with VIP (Aviptadil) showed reduced pulmonary vascular resistance and improved stroke volume (Petkov et al., 2003; Leuchte et al., 2008), additional trials showed no benefit (Said, 2012). Future work needs to fully dissect VPAC1, VPAC2 and PAC1 receptor dependent signalling in the pulmonary circulation and develop more specific and stable agonists that can be tested in the clinic: conjugating VIP to nanoparticles or co-administering of VIP with a neutral endopeptidases inhibitor has been shown to prevent VIP degradation and augment its effects (Leuchte et al., 2015; Athari et al., 2016).

Endothelin Receptors

Endothelin (ET-1), which is produced and released predominantly by PAEC, is crucial for regulating pulmonary vascular tone and seen as a key mediator of PAH (Abman, 2009). ET-1 mediates its action via two ET receptor subtypes: ET_A and ET_B, which are $G\alpha_q$-coupled. PAEC express both ET_A and ET_B receptors, whereas PASMC predominately express ET_A (Table 1). ET_B activation in PAEC promotes vasodilation by increased production of NO and PGI_2 release, inhibits apoptosis and mediates the clearance of ET-1 (Hirata et al., 1993). In contrast, ET_A and ET_B activation in PASMC induces vasoconstriction of the pulmonary arteries (MacLean *et al.,* 1994). Elevated levels of ET-1 is observed in plasma and lungs of patients with PAH, and there is a direct correlation between ET-1 concentrations and increased pulmonary vascular resistance (Giaid et al., 1993; Bauer, 2002). The expression and distribution of both ET_A and ET_B receptors are increased in the PAH-PASMC and increased ET_A mediated vasoconstriction has been shown in both the large and small pulmonary arteries (Li et

al., 1994); increased ET_A and ET_B in PASMC contributes to increased tone of the PAH-PASMC (MacLean *et al.,* 1994). Dual endothelin receptor antagonists (ERAs), such as bosentan, are approved for PAH and shown to improve time to clinical worsening (Rubin et al., 2002). Drugs selective to the ET_A receptors were developed in order to preserve ET_B receptor endothelial-dependent vasodilation and ET-1 clearance, while inhibiting vasoconstriction and proliferation mediated by the ET_A receptors: PAH is more severe in ET_B deficient rats (Ivy et al., 2002; Wilkins, 2004). Ambrisentan, a potent ET_A antagonist improves exercise capacity and haemodynamics and is utilised for initial combination therapy although longer studies are required to assess effect on mortality. Recent crystal structure identification of the ET receptor may help facilitate new rational drug design (Shihoya et al., 2016). Of interest, functional autoantibodies for ET-1 have been shown to circulate and contribute to pathophysiology of disease by stimulating the receptor and have been associated with scleroderma induced PAH (Becker et al., 2014). Neutralisers of these autoantibodies have been implicated as a viable treatment, which could be therapeutically relevant for PAH in the future.

5-HT receptors:

The neurotransmitter 5-HT (serotonin), which is synthesised in PAEC from L-trytophan by tryptophan hydroxylase, is a potent pulmonary vasoconstrictor and mitogen that increases pulmonary artery remodelling and increases pulmonary vascular resistance (MacLean and Dempsie, 2010). PAH patients exhibit elevated levels of serotonin in plasma (Hervé et al., 1995) and increased hypoxia-induced vascular tone and remodelling can be enhanced by serotonin (Eddahibi et al., 2000). Seven 5-HT receptor families, six of which are GPCRs, mediate the response to serotonin; $5\text{-}HT_{1B}$ ($G\alpha_i$), $5\text{-}HT_{2A}$ ($G\alpha_q$), $5\text{-}HT_{2B}$ ($G\alpha_q$) and $5\text{-}HT_7$ ($G\alpha_s$) have shown to be expressed in the pulmonary circulation (Ullmer et al., 1995; Morecroft and MacLean, 1998): $5\text{-}HT_{1B}$ and $5\text{-}HT_{2B}$, both of which would increase $[Ca^{2+}]_i$ are upregulated in biopsies from PAH patients and animal models of the disease (Launay et al., 2002). $5\text{-}HT_{1B}$ receptor is a key mediator of serotonin-induced vasoconstriction and proliferation in small and large human pulmonary arteries: RhoA activation and subsequent nuclear translocation of phosphorylated ERK1/2 and activity of GATA4 are key downstream pathways in PASMC activated by serotonin (Hoyer et al., 1994; MacLean et al., 1996). Inhibition of the $5\text{-}HT_{1B}$ receptor expression ($5\text{-}HT_{1B}^{-/-}$ mice) or activity ($5\text{-}HT_{1B}$ antagonist-

GR127935) attenuated the chronic hypoxia associated vascular remodelling (Keegan et al., 2001). 5-HT_{1B} receptor antagonists have begun uncovering novel serotonin signalling pathways that elucidate the aberrant redox signalling in PAH remodelling (Hood et al., 2017). 5-HT_{2B} KO mice are protected from the development of hypoxia-induced PAH and antagonists prevent pulmonary remodelling, which highlights this receptor as an additional target (Launay et al., 2002; Blanpain et al., 2003; West et al., 2016). Furthermore, 5-HT_{2A} receptors mediate contraction and proliferation of PASMC via a $G\alpha_q$-mediated increase $[Ca^{2+}]_i$ and PKC activation (MacLean et al., 2000b), although only at serotonin concentrations above the normal physiological range; 5-HT_{2A} inhibits K_V and $hK_V1.5$ currents (Morecroft et al., 1999; MacLean et al., 2000b; Cogolludo, 2006). Unfortunately, 5-HT receptor antagonists such as PRX-8006 (5HT2B-anatgonist), ketanserin (5-HT_{2A}-anatagonist) or terguride (dual 5-$HT_{2A/B}$ antagonist) have not shown much success clinically, with studies either having to be discontinued, due to the lack of specificity of these receptors to the pulmonary circulation; 5-HT_{2A} mediates systemic vasoconstriction (McGoon and Vlietstra, 1987; Dumitrascu et al., 2011).

One additional aspect of 5-HT receptors, which still makes them relevant targets for PAH, is that their expression or function can regulate or be regulated by known risk factors of PAH. Appetite suppressants, a pharmacological risk factor for PAH, can increase serotonin and a dexfenfluramine metabolite is an agonist of 5-HT_{2A} and 5-HT_{2B} (Eddahibi et al., 2001; MacLean et al., 2004). The sex hormone oestrogen, via decreasing miR96, upregulates the $5HT_{1B}$ receptor, which implicates this GPCR in the female predominance of PAH (White et al., 2011). A mutation in 5-HT_{2B} receptor itself, which reduces nitric oxide synthase activation, has been reported in PAH, however more importantly 5-HT receptor mediated signalling has been shown to interact with BMPR-2 signalling (mutations in which underlie most cases of heritable PAH) (Dempsie and MacLean, 2008). Serotonin-mediated pulmonary remodelling and vasoconstriction is enhanced in BMPR2 deficient mice (Long et al., 2006). Serotonin inhibits BMPR2 mediated Smad1/5 and Id3 activation to increase pulmonary artery remodelling (Long et al., 2006; West et al., 2016). Fully elucidating the role of 5-HT receptors in the predisposition to PAH may highlight novel pathways leading to the development of the disease. More recently the field has moved onto targeting tryptophan hydroxylase (TPH1), or the serotonin transporter (MacLean et al., 2004;

Morecroft et al., 2007), however new pharmacological developments such as new selective antagonists, multi-receptor antagonists or even negative allosteric modulators of the 5-HT receptors could restore their potential clinical utility.

Angiotensin II and MAS receptors:

The renin angiotensin system (RAS) is a key regulator of vascular endothelial function and has been implicated in the remodelling of the pulmonary artery and the right ventricle seen with PAH (Morrell *et al.,* 1995). Increased renin, angiotensin converting enzyme (ACE) and angiotensin II (Ang II) have all been associated with PAH (Morrell et al., 1999; De Man et al., 2012a). The actions of Ang II is mediated by angiotensin receptors 1 (AT_1, $G\alpha_{q/11}$) and 2 (AT_2, $G\alpha_i$); AT_1 expression is increased with PAH, whereas AT_2 is decreased (Morrell *et al.,* 1999, De Man et al., 2012). AT_1 enhances the proliferation of PASMC via $G\alpha_{q/11}$ dependent activation of MAPK, receptor tyrosine kinases, non-receptor tyrosine kinase and increasing reactive oxygen species (Morrell et al., 1999; Heeneman et al., 2000; Mehta and Griendling, 2007). Although AT_1 antagonists, such as losartan, has been shown to prevent the progression of MCT-induced PAH (De Man et al., 2012a), their clinical utility is controversial, due to systemic side effects. In contrast AT_2 activation, due to their expression in PAEC, counteracts this proliferation by increased NO and prostacyclin production. AT_2 agonists have been shown to reduced pulmonary artery pressure, fibrosis, inflammation and improve right ventricular function in experimental models of PAH, however their response may be blunted due to the decrease in AT_2 expression with the disease (Bruce et al., 2015).

ACE-2, a more recently discovered component of the RAS system, has also been identified as a novel target in PAH (Shenoy et al., 2010). ACE-2 catalyses ANG-II to ANG 1-7, which acts on the MAS1 (class A orphan GPCR). MAS1 is expressed in PAEC and similar to AT_2 receptors can counter act the proliferative and vasoconstrictive of the ACE-ANG-II-AT_1 axis (Shenoy et al., 2010); MAS1 is downregulated with PAH. Treatment with Ang (1-7) prevented the remodelling of the pulmonary artery and right ventricular hypertrophy in a model of PAH, an effect that could be blocked by the MAS inhibitor A-779 (Shenoy et al., 2010). Recently it has been shown that the beneficial effects of AT_2 receptor agonists, such as C21, may also be through ACE2-Ang-(1-7)-Mas axis since it increases ACE2 expression and its

beneficial effects in PAH were blocked in part by A-7799. These data suggests that the RAS is worth revisiting as a therapeutic option for PAH.

Apelin receptor

More recently the Apelin receptor (APJ), a GPCR with a similar sequence to AT_1, has been identified to be an important in regulator of cardiovascular physiology and play a role in the pathophysiology of PAH (Tatemoto et al., 1998; Japp et al., 2008; Kim, 2014). The endogenous ligands of APJ receptors, which are highly expressed in PAEC, are apelin family of peptides and Elabela/Toddler (ELA); both apelin and ELA have a comparable cardiovascular profile (Kang et al., 2013; Yang et al., 2017). Agonist induced APJ signalling in PAEC activates both a G-protein dependent ($G\alpha_q$- and $G\alpha_i$-coupled) decrease in cAMP and increase in PKC activity and G-protein independent induction of β-arrestin (Yang et al., 2017). Apelin-APJ leads to pulmonary vasodilation, at least in part, by increasing endothelial NO via AMPK and Kruppel-like factor 2 (KLF2) (Chandra *et al.*, 2011; Yang *et al.,* 2015). PAH-Patients and animal models of the disease have lower levels of apelin, ELA and APJ receptors, inhibiting their ability to counteract pulmonary vasoconstriction (Yang et al., 2017). Exogenous apelin ([Pyr1]Apelin-13) and ELA (ELA-13) peptides have been shown to reverse MCT-induced remodelling of the PA and right ventricular hypertrophy, (Falcão-Pires et al., 2009). Apelin infusion during right heart catheterisation increases cardiac output and decreases pulmonary vascular resistance in patients with PAH, which supports further investigation into the therapeutic relevance of the APJ receptor in PAH (Brash et al., 2015). Apelin restores BMPR2 signalling and PAEC function, making enhancing APJ receptor signalling an attractive target for heritable PAH (Alastalo et al., 2011). An interesting aspect of APJ receptor pharmacology, is the availability of biased agonists, such as CMF-019. GPCR biased agonists have been used successfully to increase the beneficial effects of targeting GPCRs, but blunt side effects. CMF-019 decreases cAMP (G protein dependent signalling) but does not induce β-arrestin mediated internalisation (G protein independent signalling) (Read et al., 2016). Ligand dependent trafficking of the APJ receptor, mediated via β arrestin, also contributes to differential signalling pathways and cellular functions, (Lee et al., 2010a; Pope et al., 2016). Additional APJ ligand dependent signalling and trafficking could prevent

receptor downregulation with chronic agonist use and thereby be harnessed to increase the responsiveness of PAH patients to APJ targeted drugs.

Additional GPCRs, whose expression is increased in PAH-PASMC

It is clear that altered GPCR expression associated with PAH, shifts the balance of $G\alpha_i/G\alpha_q/G\alpha_{12/13}$ vs. $G\alpha_s$ signalling, favouring vasoconstriction and proliferation of PASMC. In addition to those GPCRs discussed in detail above, the expression of the Calcium sensing receptor (CaS), the Sphingosine 1-phosphate receptor 2 ($S1P_2$), the α_1-adrenoreceptor (α_1-AR) and the protease-activated receptors ($PAR_{1/2/3}$) are also increased with PAH and are important regulators of PASMC vasoconstriction, proliferation, migration and pulmonary vascular tone (Eckhart *et al.*, 1996; Hsiao *et al.*, 2005; Sacks *et al.*, 2008; Szczepaniak *et al.,* 2010; Yamamura *et al.*, 2012; Boe and Simonsson, 1980; Garcia *et al.*, 1995; Birker-Robaczewska *et al.*, 2008; Molostvov *et al.*, 2008). Upregulation of these $G\alpha_q/G\alpha_{12/13}/G\alpha_i$- coupled receptors results in $[Ca^{2+}]_i$/PKC or ERK activation and decreased cAMP accumulation (Nakaki et al., 1990; Birker-Robaczewska et al., 2008; Sacks et al., 2008; Li et al., 2011). Animal studies used to dissect the functional impact of CaSR, SIP_2R and PAR_2 show that inhibiting the expression or function of the receptors attenuates or blocks the development of experimental PAH (Kwapiszewska et al., 2012; Chen et al., 2014b; Tang et al., 2016). Advances in pharmacology have allowed for the rational design of modulators (see Table 2) for these receptors, which by shifting the balance away from vasoconstriction and proliferation, could one day have clinical utility in PAH.

Summary: The future of GPCRs in PAH

GPCRs by modulating second messengers, are important regulators of basal pulmonary vascular tone. Altered expression GPCRs and endothelial dysfunction shifts the balance of $G\alpha_i/G\alpha_q/G_{12/13}$ vs. $G\alpha_s$-dependent signalling, favouring vasoconstriction and proliferation of PASMC. Decreased $G\alpha_q$-coupled GPCRs in the endothelium and increased $G\alpha_i/G\alpha_q/G_{12/13}$- coupled GPCRs in PASMC is clearly associated with PAH (Figure 2), highlighting that altered expression of GPCRs is functionally relevant.

In addition to contributing to the imbalance in pulmonary vascular tone, GPCRs are also associated with risk factors of PAH (sex, drug/toxin exposure and crosstalk with signalling pathways responsible for the genetic predisposition), which further enforces their importance in the progression of the disease. Interestingly, sex differences have been shown in the responsiveness of PAH-patients to GPCR agonists; females respond better to ET-1 receptor antagonists and prostacyclin analogues, however the reason for this is not fully understood (Marra et al., 2016). Recently there has been extensive research into the role of the female sex hormone, oestrogen, and its metabolites in the progression of PAH; GPER (GPCR that mediates the non-genomic effects of oestrogen) is a novel target for PAH. The expression of GPER has been confirmed in PAEC and PASMC and a GPER agonist has been shown to prevent pulmonary artery remodelling and right ventricular dysfunction in MCT-induced PAH, however the mechanism and site of action is still unclear (Alencar et al., 2017). Investigating the impact of sex on GPCR expression and function in cells, such as PASMC, could be important in uncovering additional targets in the female bias of the disease and differential response to drugs. Sex-specific transcriptional profiles are evident in cultured cells and tissue (Shah *et al.,* 2014). Our preliminary studies in isolated PASMC have shown female bias of a number of previously unrecognised GPCRs, which together could differentiate the control of pulmonary vascular tone between the sexes.

Although we have focused this review on GPCR targets that mediate PASMC-dependent remodelling of the pulmonary artery with PAH, inflammation, adventitial thickening and right ventricular hypertrophy also characterise the disease. Right ventricular function is a key determinant of PAH severity and prognosis (Sandoval et al., 1994; van de Veerdonk et al., 2011). Altered adrenergic receptor expression has been shown in the right ventricle of animal models of PAH: α_1, β_1 and β_2-adrenoreceptors are decreased. Low-dose noradrenaline, via β_1-adrenoreceptors, increases right ventricular contractility, right ventricle-pulmonary artery coupling and cardiac output (Packer and Leier, 1987; Kerbaul et al., 2004). However, the effectiveness of these drugs could be blunted in the right ventricle due to downregulation of these receptors (Maron and Leopold, 2015). More recently improvement in right ventricular function and remodelling by blockade of the adrenergic receptors is gaining support (Bogaard *et al.*, 2010). β-blockers such as

bisprolol and carvedilol have also successfully improved and reversed right ventricular function and remodelling in MCT-induced PAH (De Man *et al.*, 2012; Perros *et al.*, 2017), although these are still currently contraindicated for clinical use (Galiè et al., 2016). Since the structural changes in the pulmonary artery with PAH can also be attributed to proliferative, apoptosis-resistant and migratory myofibroblasts in the adventitia, the GPCRs expressed and functional in these cells could also be useful pharmacological targets for the disease. To date a number of GPCRs, including APJ, $P2Y_2$, $A2_A$, LTB_4, $5\text{-}HT_{2A}$ and ETRs have been shown to regulate pulmonary fibroblast phenotype and fibrosis (Chen et al., 2014a; Kim, 2014; Qian et al., 2015). In addition, altered expression and function of a number of GPCRs have also been documented in a number of the inflammatory cells that infiltrate the pulmonary artery in PAH; CCR1, CCR5, CCR7, CX3CR1 and CXCR4 are targets for to inhibit the inflammation associated with the disease (Balabanian et al., 2002; Bull et al., 2004; Montani et al., 2011; Rabinovitch et al., 2014). Uncovering GPCRs, which also modulate right ventricular function, inflammation and fibroblast activation is an important direction for future therapeutic targets.

Changes in GPCR activity and expression at the cellular level associated with PAH, as outlined above and through work undertaken by our lab, correlates with altered signalling and the progression of the disease. However, it is important to acknowledge that a number of aspects of GPCR pharmacology may increase the complexity of their physiological role; constitutive activity of GPCRs, receptor desensitisation, the significance of their ability to modulate more than one signalling pathway, the stoichiometry of the pathway and their localisation in membrane microdomains need to be explored to understand their true therapeutic potential in the setting of PAH. The relative importance of G protein–dependent vs. G protein–independent pathways on GPCR activation in PASMC needs to be fully dissected. GPCRs can couple to multiple Gα proteins and can also signal via MAPK, src and β arrestin. For example, several GPCRs expressed in PASMC, such as AT_1, ET_A and $P2Y_2$, have all been shown initiate cell migration and proliferation via β arrestin, independent of their respective G protein, however such data is not available in PASMC (Morris et al., 2012; Kendall et al., 2014): highlighting the signalling pathways downstream of GPCR activation that are necessary and sufficient for their beneficial effects could provide a number of additional targets for PAH. Furthermore, we have previously shown that the cellular

localisation of GPCRs and their signalling components, for example in lipid rich microdomains such as caveolae, is important for their physiological response (Ostrom and Insel, 2004). Since a number of these microdomains are increased in PAH-PASMC (Patel et al., 2007), this could alter the response of GPCR agonists in diseased cells. For example, increased caveolae in PASMC could bring specific channels and GPCRs closer together and thereby contribute to heightened tone in the pulmonary artery; co-localisation of $K_v1.5$ and $5\text{-}HT_{2A}$ in caveolae leads to 5-HT-dependent inhibition of K_v current (Cogolludo et al., 2006). Altered GPCR localisation with PAH remains to be fully explored.

Since the intracellular level, duration and function of second messengers is governed by an array of mediators downstream of GPCRs, it may be that in order to see the full beneficial effect of a GPCR these components also need to be targeted. This is likely true for PAH, where the activity and expression of a number of phosphodiesterases (PDEs), the main enzymes responsible for the degradation of cAMP, are increased (Maclean et al., 1997; Murray et al., 2007): PDE inhibitors could additively or synergistically increase the duration and degree of response to GPCR drugs that raise cAMP. Other components of the cAMP pathway such as adenylyl cyclases, multi-drug resistant protein 4 and 5, A-kinase anchor proteins and cAMP downstream targets are also dysregulated with PAH (Jourdan et al., 2001; Ostrom et al., 2002; Hara et al., 2011). A comprehensive analysis of the expression and activity of the various components of GPCR signalling pathways could uncover a series of diagnostic markers and/or targets for PAH. A pathway dependent approach to restore second messenger signalling in a pulmonary specific manner is the way forward to developing a successful therapeutic approach for the disease.

In summary, research into GPCRs in PAH have led to a better understanding of the complexity and multi-faceted nature of the disease. Advances in GPCR pharmacology, such as allosteric modulators, biased agonists or neutralisers of autoantibodies, may offer a fresh approach to the therapeutic utility of the GPCRs shown to be successful pre-clinically (Table 2). Although major therapeutic advances have been made in the past 20 years with regard to PAH treatment, in part due to the approved drugs outlined above, new pulmonary specific targets are still required. As the field of GPCRs in PAH moves forward it is important to remember that although a number have already been identified, since individual cells can express greater than a 100 different GPCRs, it is

likely that many more associated with PAH are yet to be uncovered. New techniques identifying previously uncharacterised or even orphan GPCRs in cells have proven successful in providing insights into the pathophysiology of disease and identify vast array of new therapeutic targets (Insel et al., 2015). We have used a GPCR real-time-PCR array to profile GPCR expression in male and female control-PASMC and PAH-PASMC, which has uncovered "novel" (normally expressed but not previously recognized) GPCRs in PASMC, including orphan GPCRs (Insel *et al.*, 2015; unpublished data). Given this caveat, we believe that key GPCRs involved in "tipping the balance of pulmonary vascular tone" have not yet been investigated and could offer promising new therapeutic targets for PAH.

Acknowledgements:

This work was supported by grants from the ATS and Wellcome Trust

We have no conflict of interest to declare

FIGURES AND GRAPHS

Table 1- GPCR expression and function in pulmonary vascular cells [pulmonary artery smooth muscle cells (PASMC) and endothelial cells (PAEC)]

Table 2- GPCRs targeted clinically/ pre-clinically in PAH

Figure 1- G-protein-coupled-receptor (GPCR)-mediated signalling in pulmonary artery smooth muscle cells (PASMC). *Gα and βγ subunits dissociate upon receptor activation and initiate signalling. Additionally, recruitment of β-arrestins can also initiate G-protein independent signalling and trafficking. $G\alpha_s$ stimulates the production of cyclic AMP (cAMP) via adenylyl cyclase (AC), leading to the activation of protein kinase A (PKA) and Exchange protein directly activated by cAMP (Epac), thus vasodilating PASMCs and decreasing proliferation. $G\alpha_i$ activation inhibits AC activity thereby reducing cAMP, which in turn leads to PASMC vasoconstriction and proliferation. $G\alpha_q$ activation promotes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) generating intracellular messengers 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG activates Protein Kinase C (PKC) while IP_3 stimulates intracellular release of Ca^{2+} which then form a complex with a Ca^{2+} binding protein- calmodulin. $G\alpha_{12/13}$ activation increases RhoGEF and Rho kinase (ROCK) further promoting vasoconstriction.*

Figure 2- *GPCRs that are dysregulated in pulmonary arterial smooth muscle cells (PASMC) in patients or animal models of PAH. (Red)- $G\alpha_i$/$G\alpha_q$/$G_{\alpha 12/13}$-coupled GPCRs that have been implicated in PASMC vasoconstriction and proliferation through. (Blue) $G\alpha_s$-coupled GPCRs that have been implicated in PASMC vasodilation and reduced proliferation. Note- MAS and APJ receptors have also been reportedly downregulated in PAH, however in PAEC.*

REFERENCES

Abman, S.H. (2009). Role of Endothelin Receptor Antagonists in the Treatment of Pulmonary Arterial Hypertension. Annu. Rev. Med. *60*: 13–23.

Ahmed, M., VanPatten, S., Lakshminrusimha, S., Patel, H., Coleman, T.R., and Al-Abed, Y. (2016). Effects of novel muscarinic M3 receptor ligand C1213 in pulmonary arterial hypertension models. Physiol. Rep. *4*: e13069.

Alastalo, T.-P., Li, M., Jesus Perez, V. de, Pham, D., Sawada, H., Wang, J.K., et al. (2011). Disruption of PPARγ/β-catenin–mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. J. Clin. Invest. *121*: 3735–3746.

Alencar, A.K., Montes, G.C., Montagnoli, T., Silva, A.M., Martinez, S.T., Fraga, A.G., et al. (2017). Activation of GPER ameliorates experimental pulmonary hypertension in male rats. Eur. J. Pharm. Sci. *97*: 208–217.

Alencar, A.K.N., Pereira, S.L., Montagnoli, T.L., Maia, R.C., Kümmerle, A.E., Landgraf, S.S., et al. (2013). Beneficial effects of a novel agonist of the adenosine A 2A receptor on monocrotaline-induced pulmonary hypertension in rats. Br. J. Pharmacol. *169*: 953–962.

Ancellin, N., and Hla, T. (1999). Differential pharmacological properties and signal transduction of the sphingosine 1-phosphate receptors EDG-1, EDG-3, and EDG-5. J. Biol. Chem. *274*: 18997–9002.

Asnicar, M.A., Köster, A., Heiman, M.L., Tinsley, F., Smith, D.P., Galbreath, E., et al. (2002). Vasoactive Intestinal Polypeptide/Pituitary Adenylate Cyclase-Activating Peptide Receptor 2 Deficiency in Mice Results in Growth Retardation and Increased Basal Metabolic Rate. Endocrinology *143*: 3994–4006.

Athari, S.S., Pourpak, Z., Folkerts, G., Garssen, J., Moin, M., Adcock, I.M., et al. (2016). Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur. J. Pharmacol. *791*: 811–820.

Balabanian, K., Foussat, A., Dorfmüller, P., Durand-Gasselin, I., Capel, F., Bouchet-Delbos, L., et al. (2002). CX 3 C Chemokine Fractalkine in Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *165*: 1419–1425.

Barnes, P.J., and Liu, S.F. (1995). Regulation of pulmonary vascular tone. Pharmacol. Rev. *47*: 87–131.

Barst, R.J., Langleben, D., Frost, A., Horn, E.M., Oudiz, R., Shapiro, S., et al. (2004). Sitaxsentan Therapy for Pulmonary Arterial Hypertension. *169*: 441–447.

Barst, R.J., Rubin, L.J., Long, W.A., McGoon, M.D., Rich, S., Badesch, D.B., et al. (1996). A Comparison of Continuous Intravenous Epoprostenol (Prostacyclin) with Conventional Therapy for Primary Pulmonary Hypertension. N. Engl. J. Med. *334*: 296–301.

Bauer, M. (2002). Selective Upregulation of Endothelin B Receptor Gene Expression in Severe Pulmonary Hypertension. Circulation *105*: 1034–1036.

Becker, M.O., Kill, A., Kutsche, M., Guenther, J., Rose, A., Tabeling, C., et al.

(2014). Vascular Receptor Autoantibodies in Pulmonary Arterial Hypertension Associated with Systemic Sclerosis. Am. J. Respir. Crit. Care Med. *190*: 808–817.

Birker-Robaczewska, M., Studer, R., Haenig, B., Menyhart, K., Hofmann, S., and Nayler, O. (2008). bFGF induces S1P 1 receptor expression and functionality in human pulmonary artery smooth muscle cells. J. Cell. Biochem. *105*: 1139–1145.

Blanpain, C., Poul, E. Le, Parma, J., Knoop, C., Detheux, M., Parmentier, M., et al. (2003). Serotonin 5-HT(2B) receptor loss of function mutation in a patient with fenfluramine-associated primary pulmonary hypertension. Cardiovasc. Res. *60*: 518–28.

Boe, J., and Simonsson, B.G. (1980). Adrenergic receptors and sympathetic agents in isolated human pulmonary arteries. Eur. J. Respir. Dis. *61*: 195–202.

Bogaard, H.J., Natarajan, R., Mizuno, S., Abbate, A., Chang, P.J., Chau, V.Q., et al. (2010). Adrenergic receptor blockade reverses right heart remodeling and dysfunction in pulmonary hypertensive rats. Am. J. Respir. Crit. Care Med. *182*: 652–660.

Bonvallet, S.T., Zamora, M.R., Hasunuma, K., Sato, K., Hanasato, N., Anderson, D., et al. (1994). BQ123, an ETA-receptor antagonist, attenuates hypoxic pulmonary hypertension in rats. Am. J. Physiol. *266*: H1327-31.

Boomsma, J.D., Foo, H.D., and Said, S.I. (1991). Vasoactive intestinal peptide (VIP) reverses endothelin-induced contractions of guinea-pig trachea and pulmonary artery. 273–277.

Brash, L., Barnes, G., Brewis, M., Church, C., Gibbs, S., Howard, L., et al. (2015). Apelin improves cardiac output in patients with pulmonary arterial hypertension. Eur. Respir. J. *46*:.

Bruce, E., Shenoy, V., Rathinasabapathy, A., Espejo, A., Horowitz, A., Oswalt, A., et al. (2015). Selective activation of angiotensin AT 2 receptors attenuates progression of pulmonary hypertension and inhibits cardiopulmonary fibrosis. Br. J. Pharmacol. *172*: 2219–2231.

Bull, T.M., Coldren, C.D., Moore, M., Sotto-Santiago, S.M., Pham, D. V., Nana-Sinkam, S.P., et al. (2004). Gene Microarray Analysis of Peripheral Blood Cells in Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *170*: 911–919.

Busto, R., Prieto, J.C., Bodega, G., Zapatero, J., and Carrero, I. (2000). Immunohistochemical localization and distribution of VIP/PACAP receptors in human lung. Peptides *21*: 265–269.

Chandra, S.M., Razavi, H., Kim, J., Agrawal, R., Kundu, R.K., Jesus Perez, V. de, et al. (2011). Disruption of the Apelin-APJ System Worsens Hypoxia-Induced Pulmonary Hypertension. Arterioscler. Thromb. Vasc. Biol. *31*: 814–820.

Chattergoon, N.N., D'Souza, F.M., Deng, W., Chen, H., Hyman, A.L., Kadowitz, P.J., et al. (2005). Antiproliferative effects of calcitonin gene-related peptide in aortic and pulmonary artery smooth muscle cells. Am. J. Physiol. Lung Cell. Mol. Physiol. *288*: L202-11.

Chen, C., Han, X., Fan, F., Liu, Y., Wang, T., Wang, J., et al. (2014a). Serotonin drives the activation of pulmonary artery adventitial fibroblasts and TGF-β1/Smad3-

mediated fibrotic responses through 5-HT2A receptors. Mol. Cell. Biochem. *397*: 267–276.

Chen, J., Tang, H., Sysol, J.R., Moreno-Vinasco, L., Shioura, K.M., Chen, T., et al. (2014b). The Sphingosine Kinase 1/Sphingosine-1-Phosphate Pathway in Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *190*: 1032–1043.

Chootip, K., Ness, K.F., Wang, Y., Gurney, A.M., and Kennedy, C. (2002). Regional variation in P2 receptor expression in the rat pulmonary arterial circulation. Br. J. Pharmacol. *137*: 637–646.

Christman, B.W., McPherson, C.D., Newman, J.H., King, G.A., Bernard, G.R., Groves, B.M., et al. (1992). An Imbalance between the Excretion of Thromboxane and Prostacyclin Metabolites in Pulmonary Hypertension. N. Engl. J. Med. *327*: 70–75.

Cogolludo, A. (2003). Thromboxane A2-Induced Inhibition of Voltage-Gated K+ Channels and Pulmonary Vasoconstriction: Role of Protein Kinase C. Circ. Res. *93*: 656–663.

Cogolludo, A. (2006). Serotonin Inhibits Voltage-Gated K+ Currents in Pulmonary Artery Smooth Muscle Cells: Role of 5-HT2A Receptors, Caveolin-1, and KV1.5 Channel Internalization. Circ. Res. *98*: 931–938.

Cogolludo, A., Moreno, L., Lodi, F., Frazziano, G., Cobeño, L., Tamargo, J., et al. (2006). Serotonin inhibits voltage-gated K+ currents in pulmonary artery smooth muscle cells: role of 5-HT2A receptors, caveolin-1, and KV1.5 channel internalization. Circ. Res. *98*: 931–8.

Crnkovic, S., Egemnazarov, B., Jain, P., Seay, U., Gattinger, N., Marsh, L.M., et al. (2014). NPY/Y 1 receptor-mediated vasoconstrictory and proliferative effects in pulmonary hypertension. Br. J. Pharmacol. *171*: 3895–3907.

Dempsie, Y., and MacLean, M.R. (2008). Pulmonary hypertension: therapeutic targets within the serotonin system. Br. J. Pharmacol. *155*: 455–462.

Dumitrascu, R., Kulcke, C., Konigshoff, M., Kouri, F., Yang, X., Morrell, N., et al. (2011). Terguride ameliorates monocrotaline-induced pulmonary hypertension in rats. Eur. Respir. J. *37*: 1104–1118.

Eckhart, A.D., Zhu, Z., Arendshorst, W.J., and Faber, J.E. (1996). Oxygen modulates alpha 1B-adrenergic receptor gene expression by arterial but not venous vascular smooth muscle. Am. J. Physiol. *271*: H1599-608.

Eddahibi, S., Adnot, S., Frisdal, E., Levame, M., Hamon, M., and Raffestin, B. (2001). Dexfenfluramine-associated changes in 5-hydroxytryptamine transporter expression and development of hypoxic pulmonary hypertension in rats. J. Pharmacol. Exp. Ther. *297*: 148–54.

Eddahibi, S., Hanoun, N., Lanfumey, L., Lesch, K.P., Raffestin, B., Hamon, M., et al. (2000). Attenuated hypoxic pulmonary hypertension in mice lacking the 5-hydroxytryptamine transporter gene. J. Clin. Invest. *105*: 1555–1562.

Fabricius, D., Karacay, B., Shutt, D., Leverich, W., Schafer, B., Takle, E., et al. (2011). Characterization of Intestinal and Pancreatic Dysfunction in VPAC1-Null Mutant Mouse. Pancreas *40*: 861–871.

Falcão-Pires, I., Gonçalves, N., Henriques-Coelho, T., Moreira-Gonçalves, D., Roncon-Albuquerque, R., and Leite-Moreira, A.F. (2009). Apelin decreases myocardial injury and improves right ventricular function in monocrotaline-induced pulmonary hypertension. Am. J. Physiol. Heart Circ. Physiol. *296*: H2007-14.

Falcetti, E., Flavell, D.M., Staels, B., Tinker, A., Haworth, S.G., and Clapp, L.H. (2007). IP receptor-dependent activation of PPARγ by stable prostacyclin analogues. Biochem. Biophys. Res. Commun. *360*: 821–827.

Falcetti, E., Hall, S.M., Phillips, P.G., Patel, J., Morrell, N.W., Haworth, S.G., et al. (2010). Smooth Muscle Proliferation and Role of the Prostacyclin (IP) Receptor in Idiopathic Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *182*: 1161–1170.

Frishman, W.H., Huberfeld, S., Okin, S., Wang, Y.H., Kumar, A., and Shareef, B. (1995). Serotonin and serotonin antagonism in cardiovascular and non-cardiovascular disease. J. Clin. Pharmacol. *35*: 541–572.

Fuchikami, C., Murakami, K., Tajima, K., Homan, J., Kosugi, K., Kuramoto, K., et al. (2017). A comparison of vasodilation mode among selexipag (NS-304; [2-{4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-N-(methylsulfonyl)acetamide]), its active metabolite MRE-269 and various prostacyclin receptor agonists in rat, porcine and human pulmonar. Eur. J. Pharmacol. *795*: 75–83.

Galiè, N., Humbert, M., Vachiery, J.-L., Gibbs, S., Lang, I., Torbicki, A., et al. (2016). 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur. Heart J. *37*: 67–119.

Galiè, N., Humbert, M., Vachiéry, J.-L., Vizza, C.D., Kneussl, M., Manes, A., et al. (2002). Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. J. Am. Coll. Cardiol. *39*: 1496–502.

Galie, N., Olschewski, H., Oudiz, R.J., Torres, F., Frost, A., Ghofrani, H.A., et al. (2008). Ambrisentan for the Treatment of Pulmonary Arterial Hypertension: Results of the Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Efficacy (ARIES) Study 1 and 2. Circulation *117*: 3010–3019.

Garcia, J.G., Liu, F., Verin, A.D., Birukova, A., Dechert, M.A., Gerthoffer, W.T., et al. (2001). Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. J. Clin. Invest. *108*: 689–701.

Garcia, J.G., Verin, A.D., Schaphorst, K., Siddiqui, R., Patterson, C.E., Csortos, C., et al. (1999). Regulation of endothelial cell myosin light chain kinase by Rho, cortactin, and p60(src). Am. J. Physiol. *276*: L989-98.

Garcia, J.G.N., Davis, H.W., and Patterson, C.E. (1995). Regulation of endothelial cell gap formation and barrier dysfunction: Role of myosin light chain phosphorylation. J. Cell. Physiol. *163*: 510–522.

Geraci, M.W., Gao, B., Shepherd, D.C., Moore, M.D., Westcott, J.Y., Fagan, K.A., et al. (1999). Pulmonary prostacyclin synthase overexpression in transgenic mice protects against development of hypoxic pulmonary hypertension. J. Clin. Invest. *103*: 1509–1515.

Giaid, A., Yanagisawa, M., Langleben, D., Michel, R.P., Levy, R., Shennib, H., et al. (1993). Expression of Endothelin-1 in the Lungs of Patients with Pulmonary Hypertension. N. Engl. J. Med. *328*: 1732–1739.

Hamidi, S.A., Lin, R.Z., Szema, A.M., Lyubsky, S., Jiang, Y., and Said, S.I. (2011). VIP and endothelin receptor antagonist: An effective combination against experimental pulmonary arterial hypertension. Respir. Res. *12*: 141.

Hamidi, S.A.D.K.G.. M.S.. S.S.I. (2005). Pulmonary hypertension in Fawn-hooded rats: Rapid induction with alveolar hypoxia, correlation with upregulation of endothelin receptors, and attenuation by vasoactive intestinal peptide. Proc Ame Thorac. Soc. *2*:.

Hara, Y., Sassi, Y., Guibert, C., Gambaryan, N., Dorfmüller, P., Eddahibi, S., et al. (2011). Inhibition of MRP4 prevents and reverses pulmonary hypertension in mice. J. Clin. Invest. *121*: 2888–2897.

Heeneman, S., Haendeler, J., Saito, Y., Ishida, M., and Berk, B.C. (2000). Angiotensin II induces transactivation of two different populations of the platelet-derived growth factor beta receptor. Key role for the p66 adaptor protein Shc. J. Biol. Chem. *275*: 15926–32.

Hervé, P., Launay, J., Scrobohaci, M.-L., Brenot, F., Simonneau, G., Petitpretz, P., et al. (1995). Increased plasma serotonin in primary pulmonary hypertension. Am. J. Med. *99*: 249–254.

Hirata, T., and Narumiya, S. (2011). Prostanoid Receptors. Chem. Rev. *111*: 6209–6230.

Hirata, Y., Emori, T., Eguchi, S., Kanno, K., Imai, T., Ohta, K., et al. (1993). Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. J. Clin. Invest. *91*: 1367–1373.

Hood, K.Y., Mair, K.M., Harvey, A.P., Montezano, A.C., Touyz, R.M., and MacLean, M.R. (2017). Serotonin Signaling Through the 5-HT 1B Receptor and NADPH Oxidase 1 in Pulmonary Arterial HypertensionHighlights. Arterioscler. Thromb. Vasc. Biol. *37*: 1361–1370.

Hoshikawa, Y., Voelkel, N.F., Gesell, T.L., Moore, M.D., Morris, K.G., Alger, L.A., et al. (2001). Prostacyclin receptor-dependent modulation of pulmonary vascular remodeling. Am. J. Respir. Crit. Care Med. *164*: 314–8.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., et al. (1994). International Union of Pharmacology Classification of Receptors for 5-Hydroxytryptamine (Serotonin). Pharmacol. Rev. *46*: 157–203.

Hoyer, D., Hannon, J.P., and Martin, G.R. (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol. Biochem. Behav. *71*: 533–554.

Hsiao, S.-H., Constable, P.D., Smith, G.W., and Haschek, W.M. (2005). Effects of Exogenous Sphinganine, Sphingosine, and Sphingosine-1-Phosphate on Relaxation and Contraction of Porcine Thoracic Aortic and Pulmonary Arterial Rings. Toxicol. Sci. *86*: 194–199.

Huang, X., Zou, L., Yu, X., Chen, M., Guo, R., Cai, H., et al. (2015). Salidroside attenuates chronic hypoxia-induced pulmonary hypertension via adenosine A2a

receptor related mitochondria-dependent apoptosis pathway. J. Mol. Cell. Cardiol. *82*: 153–166.

Insel, P. a, Wilderman, A., Zambon, A., Snead, A., Murray, F., Aroonsakool, N., et al. (2015). GPCR Expression in Native Cells: 'Novel' endoGPCRs as Physiologic Regulators and Therapeutic Targets. Mol. Pharmacol. 1–29.

Insel, P., Snead, A., Murray, F., Zhang, L., Yokouchi, H., Katakia, T., et al. (2012). GPCR expression in tissues and cells: Are the optimal receptors being used as drug targets? Br. J. Pharmacol. *165*: 1613–1616.

Ivy, D.D., Yanagisawa, M., Gariepy, C.E., Gebb, S.A., Colvin, K.L., and McMurtry, I.F. (2002). Exaggerated hypoxic pulmonary hypertension in endothelin B receptor-deficient rats. Am. J. Physiol. - Lung Cell. Mol. Physiol. *282*: L703–L712.

Japp, A.G., Cruden, N.L., Amer, D.A.B., Li, V.K.Y., Goudie, E.B., Johnston, N.R., et al. (2008). Vascular Effects of Apelin In Vivo in Man. J. Am. Coll. Cardiol. *52*: 908–913.

Jourdan, K.B., Mason, N.A., Long, L., Philips, P.G., Wilkins, M.R., and Morrell, N.W. (2001). Characterization of adenylyl cyclase isoforms in rat peripheral pulmonary arteries. Am. J. Physiol. Cell. Mol. Physiol. *280*: L1359–L1369.

Kang, Y., Kim, J., Anderson, J.P., Wu, J., Gleim, S.R., Kundu, R.K., et al. (2013). Apelin-APJ Signaling Is a Critical Regulator of Endothelial MEF2 Activation in Cardiovascular Development. Circ. Res. *113*: 22–31.

Keegan, A., Morecroft, I., Smillie, D., Hicks, M.N., and MacLean, M.R. (2001). Contribution of the 5-HT1B Receptor to Hypoxia-Induced Pulmonary Hypertension: Converging Evidence Using 5-HT1B-Receptor Knockout Mice and the 5-HT1B/1D-Receptor Antagonist GR127935. Circ. Res. *89*: 1231–1239.

Kendall, R.T., Lee, M.H., Pleasant, D.L., Robinson, K., Kuppuswamy, D., McDermott, P.J., et al. (2014). Arrestin-dependent angiotensin AT1receptor signaling regulates Akt and mTor-mediated protein synthesis. J. Biol. Chem. *289*: 26155–26166.

Kerbaul, F., Rondelet, B., Motte, S., Fesler, P., Hubloue, I., Ewalenko, P., et al. (2004). Effects of norepinephrine and dobutamine on pressure load-induced right ventricular failure. Crit. Care Med. *32*: 1035–1040.

Kim, J. (2014). Apelin-APJ Signaling: a Potential Therapeutic Target for Pulmonary Arterial Hypertension. Mol. Cells *37*: 196–201.

Koga, M., Mizuno, Y., Watanabe, I., Kawakami, H., and Goto, T. (2014). Role of VPAC2 receptor in monocrotaline-induced pulmonary hypertension in rats. J. Appl. Physiol. *117*: 383–391.

Kwapiszewska, G., Markart, P., Dahal, B.K., Kojonazarov, B., Marsh, L.M., Schermuly, R.T., et al. (2012). PAR-2 Inhibition Reverses Experimental Pulmonary Hypertension. Circ. Res. *110*: 1179–1191.

Lau, E.M.T., Giannoulatou, E., Celermajer, D.S., and Humbert, M. (2017). Epidemiology and treatment of pulmonary arterial hypertension. Nat. Rev. Cardiol. *14*: 603–614.

Launay, J.-M., Hervé, P., Peoc'h, K., Tournois, C., Callebert, J., Nebigil, C.G., et al. (2002). Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. Nat. Med. *8*: 1129–1135.

Leblais, V., Delannoy, E., Fresquet, F., Bégueret, H., Bellance, N., Banquet, S., et al. (2008). beta-adrenergic relaxation in pulmonary arteries: preservation of the endothelial nitric oxide-dependent beta2 component in pulmonary hypertension. Cardiovasc. Res. *77*: 202–10.

Lee, D.K., Ferguson, S.S.G., George, S.R., and O'Dowd, B.F. (2010a). The fate of the internalized apelin receptor is determined by different isoforms of apelin mediating differential interaction with β-arrestin. Biochem. Biophys. Res. Commun. *395*: 185–189.

Lee, Y.H., Mungunsukh, O., Tutino, R.L., Marquez, A.P., and Day, R.M. (2010b). Angiotensin-II-induced apoptosis requires regulation of nucleolin and Bcl-xL by SHP-2 in primary lung endothelial cells. J. Cell Sci. *123*: 1634–1643.

Lefkowitz, R.J. (1993). G protein—coupled receptor kinases. Cell *74*: 409–412.

Leuchte, H.H., Baezner, C., Baumgartner, R.A., Bevec, D., Bacher, G., Neurohr, C., et al. (2008). Inhalation of vasoactive intestinal peptide in pulmonary hypertension. Eur. Respir. J. *32*: 1289–94.

Leuchte, H.H., Prechtl, C., Callegari, J., Meis, T., Haziraj, S., Bevec, D., et al. (2015). Augmentation of the effects of vasoactive intestinal peptide aerosol on pulmonary hypertension via coapplication of a neutral endopeptidase 24.11 inhibitor. Am. J. Physiol. - Lung Cell. Mol. Physiol. *308*: L563–L568.

Li, G., Wang, Q., Hao, J., Xing, W., Guo, J., Li, H., et al. (2011). The functional expression of extracellular calcium-sensing receptor in rat pulmonary artery smooth muscle cells. J. Biomed. Sci. *18*: 16.

Li, H., Chen, S.J., Chen, Y.F., Meng, Q.C., Durand, J., Oparil, S., et al. (1994). Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. J. Appl. Physiol. *77*: 1451 LP-1459.

Liu, Y., Tian, H.Y., Yan, X.L., Fan, F.L., Wang, W.P., Han, J.L., et al. (2013). Serotonin inhibits apoptosis of pulmonary artery smooth muscle cell by pERK1/2 and PDK through 5-HT1B receptors and 5-HT transporters. Cardiovasc. Pathol. *22*: 451–457.

Long, L., Maclean, M.R., Jeffery, T.K., Morecroft, I., Yang, X., Rudarakanchana, N., et al. (2006). Serotonin Increases Susceptibility to Pulmonary Hypertension in BMPR2 -Deficient Mice.

Maarman, G.J., Schulz, R., Sliwa, K., Schermuly, R.T., and Lecour, S. (2017). Novel putative pharmacological therapies to protect the right ventricle in pulmonary hypertension: a review of current literature. Br. J. Pharmacol. *174*: 497–511.

MacKenzie, C.J., Lutz, E.M., McCulloch, D.A., Mitchell, R., and Harmar, A.J. (1996). Phospholipase C activation by VIP1 and VIP2 receptors expressed in COS 7 cells involves a pertussis toxin-sensitive mechanism. Ann. N. Y. Acad. Sci. *805*: 579–84.

MacLean, M.R., Alexander, D., Stirrat, A., Gallagher, M., Douglas, S.A., Ohlstein, E.H., et al. (2000a). Contractile responses to human urotensin-II in rat and human

pulmonary arteries: effect of endothelial factors and chronic hypoxia in the rat. Br. J. Pharmacol. *130*: 201–204.

MacLean, M.R., Clayton, R.A., Templeton, A.G.B., and Morecroft, I. (1996). Evidence for 5-HT1-like receptor-mediated vasoconstriction in human pulmonary artery. Br. J. Pharmacol. *119*: 277–282.

MacLean, M.R., and Dempsie, Y. (2010). The Serotonin Hypothesis of Pulmonary Hypertension Revisited. In Advances in Experimental Medicine and Biology, (United States), pp 309–322.

MacLean, M.R., Deuchar, G.A., Hicks, M.N., Morecroft, I., Shen, S., Sheward, J., et al. (2004). Overexpression of the 5-Hydroxytryptamine Transporter Gene: Effect on Pulmonary Hemodynamics and Hypoxia-Induced Pulmonary Hypertension. Circulation *109*: 2150–2155.

MacLean, M.R., Hervé, P., Eddahibi, S., and Adnot, S. (2000b). 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. Br. J. Pharmacol. *131*: 161–8.

Maclean, M.R., Johnston, E.D., Mcculloch, K.M., Pooley, L., Houslay, M.D., and Sweeney, G. (1997). Phosphodiesterase isoforms in the pulmonary arterial circulation of the rat: changes in pulmonary hypertension. J. Pharmacol. Exp. Ther. *283*: 619–24.

MacLean, M.R., McCulloch, K.M., and Baird, M. (1994). Endothelin ETA- and ETB-receptor-mediated vasoconstriction in rat pulmonary arteries and arterioles. J. Cardiovasc. Pharmacol. *23*: 838–45.

Man, F. De, Tu, L., Handoko, L., Rain, S., Ruiter, G., François, C., et al. (2012a). Dysregulated renin-angiotensin-aldosterone system contributes to pulmonary arterial hypertension. Am. J. Respir. Crit. Care Med. *186*: 780–789.

Man, F.S. De, Handoko, M.L., Ballegoij, J.J.M. Van, Schalij, I., Bogaards, S.J.P., Postmus, P.E., et al. (2012b). Bisoprolol delays progression towards right heart failure in experimental pulmonary hypertension. Circ. Hear. Fail. *5*: 97–105.

Maron, B.A., and Leopold, J.A. (2015). Emerging Concepts in the Molecular Basis of Pulmonary Arterial Hypertension: Part II: Neurohormonal Signaling Contributes to the Pulmonary Vascular and Right Ventricular Pathophenotype of Pulmonary Arterial Hypertension. Circulation *131*: 2079–91.

Marra, A.M., Benjamin, N., Eichstaedt, C., Salzano, A., Arcopinto, M., Gargani, L., et al. (2016). Gender-related differences in pulmonary arterial hypertension targeted drugs administration. Pharmacol. Res. *114*: 103–109.

McCormack, D.G., Barnes, P.J., and Evans, T.W. (1989). Purinoceptors in the pulmonary circulation of the rat and their role in hypoxic vasoconstriction. Br. J. Pharmacol. *98*: 367–372.

Mcculloch, K.M., Docherty, C., and Maclean, M.R. (1998). Endothelin receptors mediating contraction of rat and human pulmonary resistance arteries: eect of chronic hypoxia in the rat. 1–10.

McGoon, M.D., and Vlietstra, R.E. (1987). Acute hemodynamic response to the S2-serotonergic receptor antagonist, ketanserin, in patients with primary pulmonary

hypertension. Int. J. Cardiol. *14*: 303–9.

McLaughlin, V. V., Shah, S.J., Souza, R., and Humbert, M. (2015). Management of Pulmonary Arterial Hypertension. J. Am. Coll. Cardiol. *65*: 1976–1997.

Mehta, P.K., and Griendling, K.K. (2007). Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am. J. Physiol. Cell Physiol. *292*: C82-97.

Mei, Y., Jin, H., Tian, W., Wang, H., Wang, H., Zhao, Y., et al. (2011). Urantide alleviates monocrotaline induced pulmonary arterial hypertension in Wistar rats. Pulm. Pharmacol. Ther. *24*: 386–393.

Molostvov, G., Fletcher, S., Bland, R., and Zehnder, D. (2008). Extracellular Calcium-Sensing Receptor Mediated Signalling is Involved in Human Vascular Smooth Muscle Cell Proliferation and Apoptosis. Cell. Physiol. Biochem. *22*: 413–422.

Montani, D., Perros, F., Gambaryan, N., Girerd, B., Dorfmuller, P., Price, L.C., et al. (2011). C-Kit–Positive Cells Accumulate in Remodeled Vessels of Idiopathic Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *184*: 116–123.

Morecroft, I., Dempsie, Y., Bader, M., Walther, D.J., Kotnik, K., Loughlin, L., et al. (2007). Effect of tryptophan hydroxylase 1 deficiency on the development of hypoxia-induced pulmonary hypertension. Hypertension *49*: 232–236.

Morecroft, I., Heeley, R.P., Prentice, H.M., Kirk, A., and MacLean, M.R. (1999). 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: importance of the 5-HT1B receptor. Br. J. Pharmacol. *128*: 730–734.

Morecroft, I., and MacLean, M.R. (1998). 5-Hydroxytryptamine receptors mediating vasoconstriction and vasodilation in perinatal and adult rabbit small pulmonary arteries. Br. J. Pharmacol. *125*: 69–78.

Morgan, J.M., McCormack, D.G., Griffiths, M.J., Morgan, C.J., Barnes, P.J., and Evans, T.W. (1991). Adenosine as a vasodilator in primary pulmonary hypertension. Circulation *84*: 1145–1149.

Morrell, N.W., Adnot, S., Archer, S.L., Dupuis, J., Lloyd Jones, P., MacLean, M.R., et al. (2009). Cellular and Molecular Basis of Pulmonary Arterial Hypertension. J. Am. Coll. Cardiol. *54*: S20–S31.

Morrell, N.W., Morris, K.G., and Stenmark, K.R. (1995). Role of angiotensin-converting enzyme and angiotensin II in development of hypoxic pulmonary hypertension. Am. J. Physiol. - Hear. Circ. Physiol. *269*: H1186 LP-H1194.

Morrell, N.W., Upton, P.D., Kotecha, S., Huntley, A., Yacoub, M.H., Polak, J.M., et al. (1999). Angiotensin II activates MAPK and stimulates growth of human pulmonary artery smooth muscle via AT1 receptors. Am. J. Physiol. *277*: L440-8.

Morris, G.E., Nelson, C.P., Brighton, P.J., Standen, N.B., Challiss, R.A.J., and Willets, J.M. (2012). Arrestins 2 and 3 differentially regulate ET A and P2Y 2 receptor-mediated cell signaling and migration in arterial smooth muscle. Am. J. Physiol. Physiol. *302*: C723–C734.

Morrison, K., Studer, R., Ernst, R., Haag, F., Kauser, K., and Clozel, M. (2012). Differential Effects of Selexipag and Prostacyclin Analogs in Rat Pulmonary Artery. J. Pharmacol. Exp. Ther. *343*: 547–555.

Murray, F., Patel, H.H., Suda, R.Y.S., Zhang, S., Thistlethwaite, P. a, Yuan, J.X.-J., et al. (2007). Expression and activity of cAMP phosphodiesterase isoforms in pulmonary artery smooth muscle cells from patients with pulmonary hypertension: role for PDE1. Am. J. Physiol. Lung Cell. Mol. Physiol. *292*: L294-303.

Murray, F., Yuan, J.X., and Insel, P.A. (2011). Textbook of Pulmonary Vascular Disease (Boston, MA: Springer US).

Nakaki, T., Nakayama, M., Yamamoto, S., and Kato, R. (1990). Alpha-adrenergic stimulation and beta-adrenergic inhibition of DNA synthesis in vascular smooth muscle cells. Mol.Pharmacol. *37,No.1*: 30–36.

Norel, X., Walch, L., Costantino, M., Labat, C., Gorenne, I., Dulmet, E., et al. (1996). M1 and M3 muscarinic receptors in human pulmonary arteries. Br. J. Pharmacol. *119*: 149–57.

Olschewski, A., Li, Y., Tang, B., Hanze, J., Eul, B., Bohle, R.M., et al. (2006). Impact of TASK-1 in human pulmonary artery smooth muscle cells. Circ. Res. *98*: 1072–80.

Olschewski, H., Simonneau, G., Galiè, N., Higenbottam, T., Naeije, R., Rubin, L.J., et al. (2002). Inhaled Iloprost for Severe Pulmonary Hypertension. N. Engl. J. Med. *347*: 322–329.

Ortiz, J.L., Labat, C., Norel, X., Gorenne, I., Verley, J., and Brink, C. (1992). Histamine receptors on human isolated pulmonary arterial muscle preparations: effects of endothelial cell removal and nitric oxide inhibitors. J. Pharmacol. Exp. Ther. *260*: 762–767.

Ostrom, R.S., and 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.

Ostrom, R.S., Liu, X., Head, B.P., Gregorian, C., Seasholtz, T.M., and Insel, P. a (2002). Localization of adenylyl cyclase isoforms and G protein-coupled receptors in vascular smooth muscle cells: expression in caveolin-rich and noncaveolin domains. Mol. Pharmacol. *62*: 983–992.

Otto, C. (2004). Pulmonary Hypertension and Right Heart Failure in Pituitary Adenylate Cyclase-Activating Polypeptide Type I Receptor-Deficient Mice. Circulation *110*: 3245–3251.

Packer, M., and Leier, C. V (1987). Survival in congestive heart failure during treatment with drugs with positive inotropic actions. Circulation *75*: IV55-63.

Patel, H.H., Zhang, S., Murray, F., Suda, R.Y.S., Head, B.P., Yokoyama, U., et al. (2007). Increased smooth muscle cell expression of caveolin-1 and caveolae contribute to the pathophysiology of idiopathic pulmonary arterial hypertension. FASEB J. *21*: 2970–2979.

Pedersen, K.E., Buckner, C.K., Meeker, S.N., and Undem, B.J. (2000). Pharmacological examination of the neurokinin-1 receptor mediating relaxation of human intralobar pulmonary artery. J. Pharmacol. Exp. Ther. *292*: 319–325.

Perros, F., Man, F.S. de, Bogaard, H.J., Antigny, F., Simonneau, G., Bonnet, S., et al. (2017). Use of β-Blockers in Pulmonary Hypertension. Circ. Hear. Fail. *10*: e003703.

Perros, F., Ranchoux, B., Izikki, M., Bentebbal, S., Happé, C., Antigny, F., et al. (2015). Nebivolol for Improving Endothelial Dysfunction, Pulmonary Vascular Remodeling, and Right Heart Function in Pulmonary Hypertension. J. Am. Coll. Cardiol. *65*: 668–680.

Petkov, V., Mosgoeller, W., Ziesche, R., Raderer, M., Stiebellehner, L., Vonbank, K., et al. (2003). Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. J. Clin. Invest. *111*: 1339–1346.

Pope, G.R., Tilve, S., McArdle, C.A., Lolait, S.J., and O'Carroll, A.-M. (2016). Agonist-induced internalization and desensitization of the apelin receptor. Mol. Cell. Endocrinol. *437*: 108–119.

Porvasnik, S.L., Germain, S., Embury, J., Gannon, K.S., Jacques, V., Murray, J., et al. (2010). PRX-08066, a novel 5-hydroxytryptamine receptor 2B antagonist, reduces monocrotaline-induced pulmonary arterial hypertension and right ventricular hypertrophy in rats. J. Pharmacol. Exp. Ther. *334*: 364–372.

Pulido, T., Adzerikho, I., Channick, R.N., Delcroix, M., Galiè, N., Ghofrani, H.-A., et al. (2013). Macitentan and Morbidity and Mortality in Pulmonary Arterial Hypertension. N. Engl. J. Med. *369*: 809–818.

Qian, J., Tian, W., Jiang, X., Tamosiuniene, R., Sung, Y.K., Shuffle, E.M., et al. (2015). Leukotriene B 4 Activates Pulmonary Artery Adventitial Fibroblasts in Pulmonary Hypertension. Hypertension *531*: HYPERTENSIONAHA.115.06370.

Rabinovitch, M., Guignabert, C., Humbert, M., and Nicolls, M.R. (2014). Inflammation and Immunity in the Pathogenesis of Pulmonary Arterial Hypertension. Circ. Res. *115*: 165–175.

Rajagopal, S., Rajagopal, K., and Lefkowitz, R.J. (2010). Teaching old receptors new tricks: biasing seven-transmembrane receptors. Nat. Rev. Drug Discov. *9*: 373–386.

Read, C., Fitzpatrick, C.M., Yang, P., Kuc, R.E., Maguire, J.J., Glen, R.C., et al. (2016). Cardiac action of the first G protein biased small molecule apelin agonist. Biochem. Pharmacol. *116*: 63–72.

Roberts, N. V, Keast, P.J., Brodeky, V., Oates, A., and Ritchie, B.C. (1992). The effects of oxytocin on the pulmonary and systemic circulation in pregnant ewes. Anaesth. Intensive Care *20*: 199—202.

Rossi, R., Coppi, F., Sgura, F., Monopoli, D., and Boriani, G. (2017). Acute hemodynamic effects of intravenous adenosine in patients with associated pulmonary arterial hypertension: Comparison with intravenous epoprostenol. Pulm. Pharmacol. Ther.

Rubin, L.J., Badesch, D.B., Barst, R.J., Galiè, N., Black, C.M., Keogh, A., et al. (2002). Bosentan Therapy for Pulmonary Arterial Hypertension. N. Engl. J. Med. *346*: 896–903.

Sacks, R.S., Firth, A.L., Remillard, C. V, Agange, N., Yau, J., Ko, E.A., et al. (2008). Thrombin-mediated increases in cytosolic [Ca2+] involve different mechanisms in

human pulmonary artery smooth muscle and endothelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. *295*: L1048-55.

Said, S.I. (2012). Vasoactive Intestinal Peptide in Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *185*: 786–786.

Said, S.I., Hamidi, S.A., Dickman, K.G., Szema, A.M., Lyubsky, S., Lin, R.Z., et al. (2007). Moderate Pulmonary Arterial Hypertension in Male Mice Lacking the Vasoactive Intestinal Peptide Gene. Circulation 1260–1269.

Sakai, S., Maruyama, H., Kimura, T., Tajiri, K., Honda, J., Homma, S., et al. (2016). Antagonists to endothelin receptor type B promote apoptosis in human pulmonary arterial smooth muscle cells. Life Sci. *159*: 116–120.

Sandoval, J., Bauerle, O., Palomar, a, Gómez, a, Martínez-Guerra, M.L., Beltrán, M., et al. (1994). Survival in primary pulmonary hypertension. Validation of a prognostic equation. Circulation *89*: 1733–1744.

Schermuly, R.T., Pullamsetti, S.S., Breitenbach, S.C., Weissmann, N., Ghofrani, H.A., Grimminger, F., et al. (2007). Iloprost-induced desensitization of the prostacyclin receptor in isolated rabbit lungs. Respir. Res. *8*: 4.

Shah, K., McCormack, C.E., and Bradbury, N.A. (2014). Do you know the sex of your cells? AJP Cell Physiol. *306*: C3–C18.

Shaul, P.W., Kinane, B., Farrar, M.A., Buja, L.M., and Magness, R.R. (1991). Prostacyclin production and mediation of adenylate cyclase activity in the pulmonary artery. Alterations after prolonged hypoxia in the rat. J. Clin. Invest. *88*: 447–455.

Shenoy, V., Ferreira, A.J., Qi, Y., Fraga-Silva, R.A., Díez-Freire, C., Dooies, A., et al. (2010). The Angiotensin-Converting Enzyme 2/Angiogenesis-(1–7)/Mas Axis Confers Cardiopulmonary Protection against Lung Fibrosis and Pulmonary Hypertension. Am. J. Respir. Crit. Care Med. *182*: 1065–1072.

Shichiri, M., Kato, H., Marumo, F., and Hirata, Y. (1997). Endothelin-1 as an Autocrine/Paracrine Apoptosis Survival Factor for Endothelial Cells. Hypertension *30*: 1198 LP-1203.

Shihoya, W., Nishizawa, T., Okuta, A., Tani, K., Dohmae, N., Fujiyoshi, Y., et al. (2016). Activation mechanism of endothelin ETB receptor by endothelin-1. Nature *537*: 363–368.

Simonneau, G., Barst, R.J., Galie, N., Naeije, R., Rich, S., Bourge, R.C., et al. (2002). Continuous Subcutaneous Infusion of Treprostinil, a Prostacyclin Analogue, in Patients with Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *165*: 800–804.

Simonneau, G., Torbicki, A., Hoeper, M.M., Delcroix, M., Karlócai, K., Galiè, N., et al. (2012). Selexipag: an oral, selective prostacyclin receptor agonist for the treatment of pulmonary arterial hypertension. Eur. Respir. J. *40*: 874–880.

Smith, A.M., Elliot, C.M., Kiely, D.G., and Channer, K.S. (2006). The role of vasopressin in cardiorespiratory arrest and pulmonary hypertension. QJM *99*: 127–133.

Szczepaniak, W.S., Pitt, B.R., and McVerry, B.J. (2010). S1P(2) receptor-dependent

Rho-kinase activation mediates vasoconstriction in the murine pulmonary circulation induced by sphingosine 1-phosphate. Am. J. Physiol. - Lung Cell. Mol. Physiol. *299*: L137–L145.

Tang, H., Yamamura, A., Yamamura, H., Song, S., Fraidenburg, D.R., Chen, J., et al. (2016). Pathogenic role of calcium-sensing receptors in the development and progression of pulmonary hypertension. Am. J. Physiol. - Lung Cell. Mol. Physiol. *310*: L846–L859.

Taraseviciene-Stewart, L., Scerbavicius, R., Stewart, J.M., Gera, L., Demura, Y., Cool, C., et al. (2005). Treatment of severe pulmonary hypertension: a bradykinin receptor 2 agonist B9972 causes reduction of pulmonary artery pressure and right ventricular hypertrophy. Peptides *26*: 1292—1300.

Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M.X., et al. (1998). Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem. Biophys. Res. Commun. *251*: 471–476.

Ullmer, C., Schmuck, K., Kalkman, H.O., and Lübbert, H. (1995). Expression of serotonin receptor mRNAs in blood vessels. FEBS Lett. *370*: 215–21.

Upton, P.D., Wharton, J., Coppock, H., Davie, N., Yang, X., Yacoub, M.H., et al. (2001). Adrenomedullin expression and growth inhibitory effects in distinct pulmonary artery smooth muscle cell subpopulations. Am J Respir Cell Mol Biol *24*: 170–178.

Veerdonk, M.C. van de, Kind, T., Marcus, J.T., Mauritz, G.-J., Heymans, M.W., Bogaard, H.-J., et al. (2011). Progressive Right Ventricular Dysfunction in Patients With Pulmonary Arterial Hypertension Responding to Therapy. J. Am. Coll. Cardiol. *58*: 2511–2519.

West, J.D., Carrier, E.J., Bloodworth, N.C., Schroer, A.K., Chen, P., Ryzhova, L.M., et al. (2016). Serotonin 2B Receptor Antagonism Prevents Heritable Pulmonary Arterial Hypertension. PLoS One *11*: e0148657.

White, K., Loughlin, L., Maqbool, Z., Nilsen, M., McClure, J., Dempsie, Y., et al. (2011). Serotonin transporter, sex, and hypoxia: microarray analysis in the pulmonary arteries of mice identifies genes with relevance to human PAH. Physiol. Genomics *43*: 417–37.

Whittle, B.J., Silverstein, A.M., Mottola, D.M., and Clapp, L.H. (2012). Binding and activity of the prostacyclin receptor (IP) agonists, treprostinil and iloprost, at human prostanoid receptors: Treprostinil is a potent DP1 and EP2 agonist. Biochem. Pharmacol. *84*: 68–75.

Wilkins, M.R. (2004). Selective or Nonselective Endothelin Receptor Blockade in Pulmonary Arterial Hypertension. Am. J. Respir. Crit. Care Med. *169*: 433–434.

Xu, M.H., Gong, Y.S., Su, M.S., Dai, Z.Y., Dai, S.S., Bao, S.Z., et al. (2011). Absence of the Adenosine A2A Receptor Confers Pulmonary Arterial Hypertension and Increased Pulmonary Vascular Remodeling in Mice. J. Vasc. Res. *48*: 171–183.

Yamada, T., Horiuchi, M., and Dzau, V.J. (1996). Angiotensin II type 2 receptor mediates programmed cell death. Proc. Natl. Acad. Sci. U. S. A. *93*: 156–160.

Yamamoto, K., Suzuki, T., Imamura, R., Nagano, T., Okabe, T., and Miyachi, H.

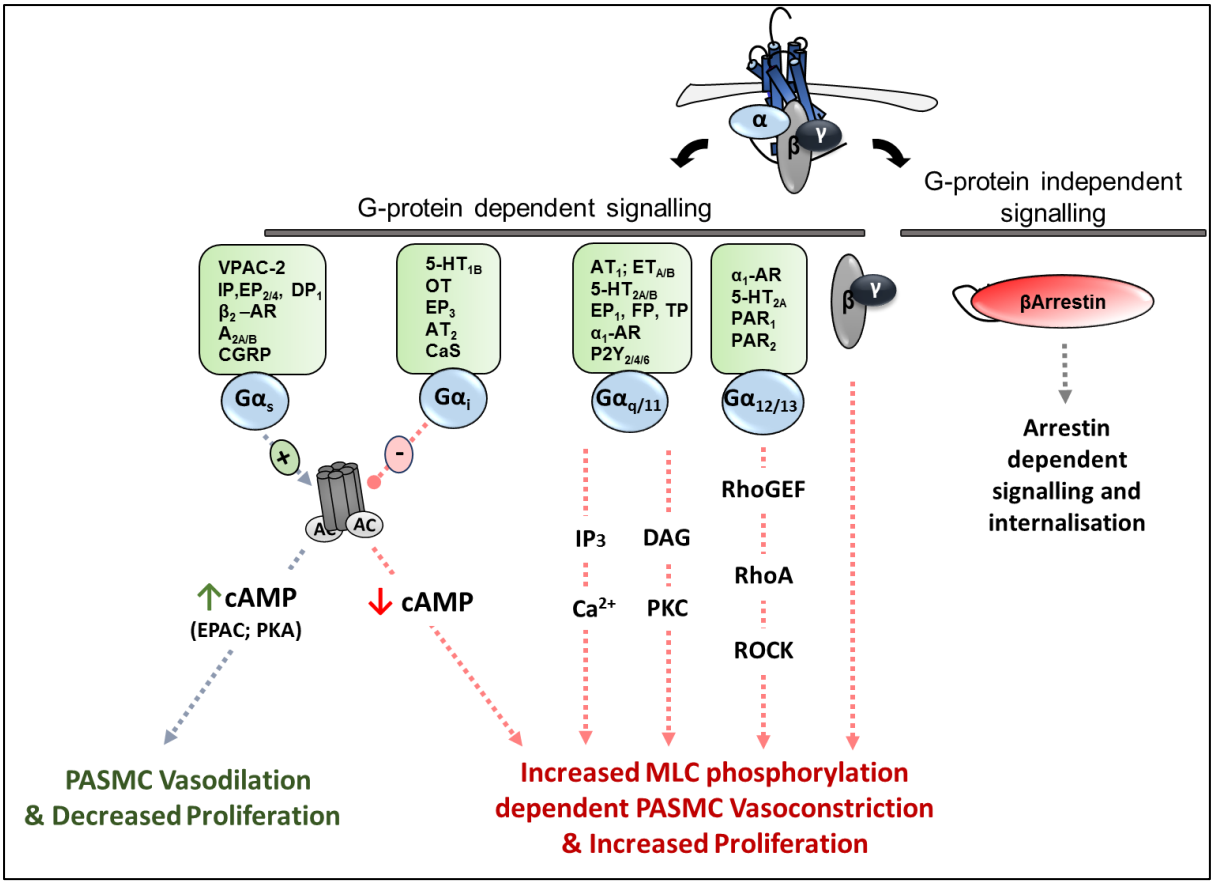
(2017). Synthesis of both enantiomers of 1,2,3,4-tetrahydroisoquinoline derivative IPPAM-1 and enantio-dependency of its positive allosteric modulation of prostacyclin receptor. Bioorg. Med. Chem. Lett. *27*: 2567–2570.

Yamamura, A., Guo, Q., Yamamura, H., Zimnicka, A.M., Pohl, N.M., Smith, K.A., et al. (2012). Enhanced Ca2+-Sensing Receptor Function in Idiopathic Pulmonary Arterial Hypertension. Circ. Res. *111*: 469–481.

Yang, P., Maguire, J.J., and Davenport, A.P. (2015). Apelin, Elabela/Toddler, and biased agonists as novel therapeutic agents in the cardiovascular system. Trends Pharmacol. Sci. *36*: 560–567.

Yang, P., Read, C., Kuc, R.E., Buonincontri, G., Southwood, M., Torella, R., et al. (2017). Elabela/Toddler Is an Endogenous Agonist of the Apelin APJ Receptor in the Adult Cardiovascular System, and Exogenous Administration of the Peptide Compensates for the Downregulation of Its Expression in Pulmonary Arterial HypertensionClinical Perspective. Circulation *135*: 1160–1173.

Zhang, S., Patel, H.H., Murray, F., Remillard, C. V, Schach, C., Thistlethwaite, P. a, et al. (2007). Pulmonary artery smooth muscle cells from normal subjects and IPAH patients show divergent cAMP-mediated effects on TRPC expression and capacitative Ca2+ entry. AJP Lung Cell. Mol. Physiol. *292*: L1202–L1210.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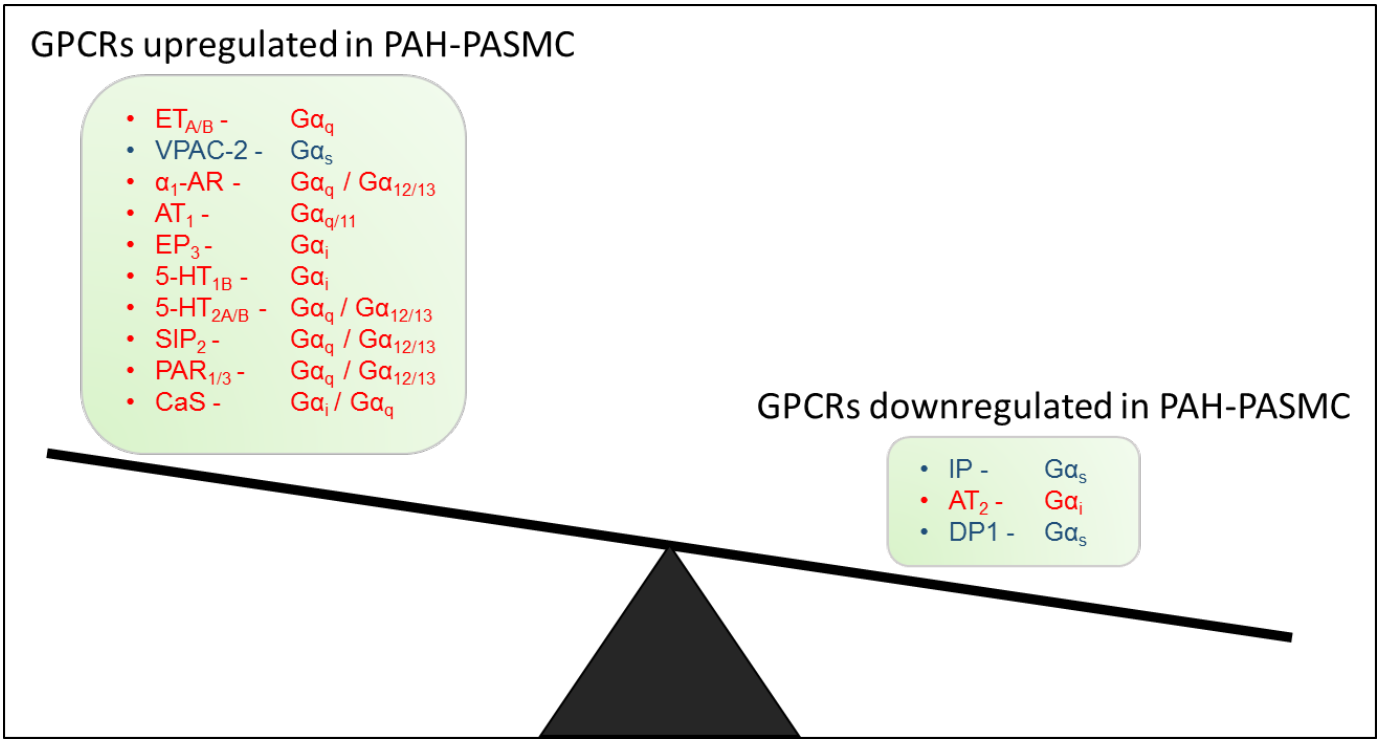
β
γ
G-protein dependent signalling
G-protein independent signalling
VPAC-2
IP,EP2/4, DP1
β2 –AR
A2A/B
CGRP
Gαs
5-HT1B
OT
EP3
AT2
CaS
Gαi
AT1; ETA/B
5-HT2A/B
EP1, FP, TP
α1-AR
P2Y2/4/6
Gαq/11
α1-AR
5-HT2A
PAR1
PAR2
Gα12/13
βArrestin
Arrestin dependent signalling and internalisation
+
-
AC
AC
↑cAMP
(EPAC; PKA)
↓ cAMP
IP3
DAG
Ca2+
PKC
RhoGEF
RhoA
ROCK
PASMC Vasodilation
& Decreased Proliferation
Increased MLC phosphorylation dependent PASMC Vasoconstriction
& Increased Proliferation


GPCRs upregulated in PAH-PASMC
• $ET_{A/B}$ - $G\alpha_q$
• VPAC-2 - $G\alpha_s$
• α_1-AR - $G\alpha_q$ / $G\alpha_{12/13}$
• AT_1 - $G\alpha_{q/11}$
• EP_3 - $G\alpha_i$
• $5\text{-}HT_{1B}$ - $G\alpha_i$
• $5\text{-}HT_{2A/B}$ - $G\alpha_q$ / $G\alpha_{12/13}$
• SIP_2 - $G\alpha_q$ / $G\alpha_{12/13}$
• $PAR_{1/3}$ - $G\alpha_q$ / $G\alpha_{12/13}$
• CaS - $G\alpha_i$ / $G\alpha_q$
GPCRs downregulated in PAH-PASMC
• IP - $G\alpha_s$
• AT_2 - $G\alpha_i$
• DP1 - $G\alpha_s$

Endogenous ligand	GPCR	Cell type	G-protein	Cell specific response	Refs
Angiotensin-II	AT_1	PASMC	$G\alpha_{q/11}$	Proliferation/ Vasoconstriction/ Anti-apoptosis	(Yamada et al., 1996; Morrell et al., 1999)
	AT_2	PAEC	$G\alpha_i$	Vasodilation/ anti-proliferation/ Apoptosis	(Lee et al., 2010; Bruce et al., 2015)
ANG 1-7	MAS	PAEC	$G\alpha_q$	Vasodilation	(Shenoy et al., 2010)
Endothelin-1	ET_A	PASMC	$G\alpha_q$	Proliferation/ Vasoconstriction	(MacLean *et al.*, 1994; Mcculloch *et al.*,1998; Shichiri et al., 1997; Sakai et al., 2016)
	ET_B	PASMC	$G\alpha_q$	Proliferation/ Vasoconstriction/ Anti-apoptosis	
	ET_B	PAEC	$G\alpha_q$	Vasodilation/ Anti-apoptosis	
Noroepinepherine / Epinepherine	β_2-AR	PASMC	$G\alpha_s$	Vasodilation	(Boe and Simonsson, 1980; Leblais et al., 2008)
	α_1-AR	PASMC	$G\alpha_q$ / $G\alpha_{12/13}$	Vasoconstriction	
	α_2-AR	PAEC	$G\alpha_q$	Vasodilation	
Acetylcholine	M_1	PAEC	$G\alpha_q$	Vasodilation	(Norel et al., 1996)
	M_3	PAEC	$G\alpha_q$	Vasodilation	
	M_3	PASMC	$G\alpha_q$	Vasoconstriction	
Bradykinin	B_2	PAEC	$G\alpha_q$	Vasodilation/ Apoptosis	(Taraseviciene-Stewart *et al.*, 2005)
Vasopressin	V1	PAEC	$G\alpha_q$	Vasodilation	(Smith et al., 2006)
Adrenomedullin	CRLR/RAMP	PASMC	$G\alpha_s$	Vasodilation	(Upton et al., 2001)
		PAEC	$G\alpha_q$	Vasodilation	
Vasoactive Intestinal Peptide	VPAC-1	PASMC	$G\alpha_s$	Vasodilation	(Busto et al., 2000)
	VPAC-2	PASMC	$G\alpha_s$	Vasodilation	
Ca^{2+}, Mg^{2+}, spermine	CaS	PASMC	$G\alpha_q$, $G\alpha_i$	Proliferation/ Vasoconstriction	(Li et al., 2011)
Calcitonin gene-related peptide	CGRP	PASMC	$G\alpha_s$	Vasodilation/ anti-proliferation	(Chattergoon et al., 2005)
	CGRP	PAEC	$G\alpha_q$		
Substance P	NK_1	PAEC	$G\alpha_q$	Vasodilation	(Pedersen et al., 2000)
	NK_2	PASMC	$G\alpha_q$	Vasoconstriction	
Histamine	H_1	PASMC	$G\alpha_q$	Vasoconstriction	(Ortiz et al., 1992)
Urotensin II	UT	PASMC	$G\alpha_q$	Vasoconstriction	(MacLean *et al.*, 2000)
Adenosine	A_{2A}	PASMC	$G\alpha_s$	Vasodilation/ Apoptosis	(Morgan et al., 1991; Xu et al., 2011; Huang et al., 2015)
	A_{2B}	PASMC	$G\alpha_s$	Vasodilation	
Oxytocin, Vasopressin	OT	PASMC	$G\alpha_i$, $G\alpha_q$	Vasoconstriction	(Roberts *et al.*, 1992; unpublished data)
ATP, ADP, UTP, UDP	$P2Y_2$, $P2Y_4$, $P2Y_6$	PASMC	$G\alpha_q$	Vasoconstriction	(McCormack *et al.*, 1989; Chootip *et al.*, 2002)
		PAEC	$G\alpha_q$	Vasodilation	

5-Hydroxytryptamine	5-HT_{1B}	PASMC	$G\alpha_i$	Proliferation/ Vasoconstriction/ Anti-apoptosis	(Morecroft and MacLean, 1998; Hoyer *et al.*, 2002; Liu et al., 2013)
	5-HT_{2A}	PASMC	$G\alpha_q$ / $G\alpha_{12/13}$	Proliferation/ Vasoconstriction/ Anti-apoptosis	
	5-HT_{2B}	PASMC	$G\alpha_q$	Proliferation/ Vasoconstriction/ Anti-apoptosis	
Prostacyclin	IP	PASMC	$G\alpha_s$	Vasodilation/ anti-proliferation	(Shaul et al., 1991)
$PGE_{1/2}$	EP_1	PASMC	$G\alpha_q$	Vasoconstriction	Hirata and Narumiya, 2011
	EP_2	PASMC	$G\alpha_s$	Vasodilation	
	EP_3	PASMC	$G\alpha_i$	Vasoconstriction	
	EP_4	PASMC	$G\alpha_s$	Vasodilation	
PGD_2	DP_1	PASMC	$G\alpha_s$	Vasodilation	
Thromboxane	FP and TP	PASMC	$G\alpha_q$	Proliferation/ Vasoconstriction	(Cogolludo, 2003)
Sphingosine	SIP_1	PAEC	$G\alpha_i$	PAEC barrier protection	(Ancellin and Hla, 1999; Garcia et al., 2001; Birker-Robaczewska et al., 2008)
	SIP_2	PASMC	$G\alpha_q$ / $G\alpha_{12/13}$	Proliferation	
	SIP_3	PAEC	$G\alpha_q$ / $G\alpha_i$ / $G\alpha_{12/13}$	PAEC barrier dysfunction	
Thrombin	PAR_1	PAEC	$G\alpha_q$ / $G\alpha_{12/13}$	PAEC barrier dysfunction	(Sacks et al., 2008)
	PAR_1/PAR_2/ PAR_3	PASMC	$G\alpha_q$ / $G\alpha_{12/13}$	Proliferation/ Vasoconstriction	
Apelin; Elabela/Toddler	APJ	PAEC	$G\alpha_q$ /$G\alpha_{i/o}$	Vasodilation	(Japp *et al.*, 2008; Yang *et al.*, 2015)
Neuropeptide Y	NPY_1	PASMC	$G\alpha_{i/o}$	Proliferation/ Vasoconstriction	(Crnkovic et al., 2014)
	NPY_2	PASMC	$G\alpha_{i/o}$	Vasoconstriction	
	NPY_4	PASMC	$G\alpha_{i/o}$	Vasoconstriction	
Oestradiol	GPER	PAEC	Gαi / Gαs	Vasodilation	(Alencar et al., 2017)
		PASMC	Gαi / Gαs	Vasodilation/ Anti-proliferative	

	GPCR	Expression In PAH	Level of endogenous ligand	Drug	Effect	Refs
Clinical/ pre-clinical Increased 6MWD and Improved PA remodelling	$ET_{A/B}$	↑	↑	Bosentan	Antagonist (clinical)	(Rubin *et al.*, 2002)
	ET_A			Ambrisentan	Antagonist (clinical)	(Galie *et al.*, 2008)
				Macitentan		(Pulido *et al.*, 2013)
				*Sitaxsentan		(Barst *et al.*, 2004)
	VPAC-2	↑	↓	Aviptadil	Agonist (clinical, in-vivo)	(Petkov *et al.*, 2003; Leuchte *et al.*, 2008)
	IP	↓	↓	Epoprostenol	Analogue (clinical)	(Barst *et al.*, 1996)
				Iloprost		(Olschewski *et al.*, 2002)
				Treprostinil		(Simonneau *et al.*, 2002)
				Beraprost		(Galiè *et al.*, 2002)
				Selexipag	Agonist (clinical)	(Simonneau *et al.*, 2012)
				MRE-269	Agonist (in-vitro, in-vivo)	(Fuchikami *et al.*, 2017)
Pre-clinical Improved Pulmonary artery remodelling & Right heart function	UT	-	-	Urantide	Antagonist (in-vivo, in-vitro)	(Mei *et al.*, 2011)
	CaS	↑	-	NPS 2143	NAM (in-vivo, in-vitro)	(Tang *et al.*, 2016)
	APJ	↓	↓	Apelin	Agonist (clinical)	(Brash *et al.*, 2015)
				Apelin/ ELA	Agonist (in-vitro, in-vivo)	(Yang *et al.*, 2017)
	A_{2B}	-	-	Adenosine	Agonist (clinical)	(Morgan *et al.*, 1991; Rossi *et al.*, 2017)
	A_{2A}	-		LASSBio-1359	Agonist (in-vitro, in-vivo)	(Alencar *et al.*, 2013)
	AT_1	↑	↑	Losartan	Antagonist (in-vitro, in-vivo)	(Morrell *et al.*, 1999; De Man *et al.*, 2012)
	AT_2	↓		C-21	Agonist (in-vitro, in-vivo)	(Bruce *et al.*, 2015)
	5-$HT1_B$	↑	↑	GR127935	Antagonist (in-vitro, in-vivo)	(Keegan *et al.*, 2001)
				SB216641		(Hood *et al.*, 2017)
	5-HT_{2A}	↑		Ketanserin	Antagonist (in-vitro)	(McGoon and Vlietstra, 1987; Frishman *et al.*, 1995)
	5-$HT_{2A/B}$			Terguride	Antagonist (in-vitro, in-vivo)	(Launay *et al.*, 2002; Dumitrascu *et al.*, 2011)
	5-HT_{2B}			PRX-08066	Antagonist (in-vitro, in-vivo)	(Porvasnik *et al.*, 2010)
	$S1P_2$	↑	↑	JTE013	Antagonist (in-vitro, in-vivo)	(Chen *et al.*, 2014)
	β_2-AR	-	-	Bisoprolol/ Carvedilol	Antagonist (in-vivo, in-vitro)	(Perros *et al.*, 2017)
				Nebivolol	Agonist (in-vivo, in-vitro)	(Perros *et al.*, 2015)
	B_2	-	-	B9972	Agonist (in-vitro, in-vivo)	(Taraseviciene-Stewart *et al.*, 2005)
	M_3	-	-	C1213	Agonist (in-vivo, in-vitro)	(Ahmed *et al.*, 2016)
	GPER	-	-	G1	Agonist (in-vitro, in-vivo)	(Alencar *et al.*, 2017)
	Mas	↓	-	ACE-2 and Ang 1-7	Agonists (in-vitro, in-vivo)	(Shenoy *et al.*, 2010)

(-) No reported data; (↑) Upregulated; (↓) Downregulated; (*) Withdrawn from clinical use; (6MWD) 6 minute walking distance; (PA) pulmonary artery; (RH) right heart.