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 (PGI₂) 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: Gαs, Gαi, Gαq/11, and Gα12/13, 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 Gαi-, Gαq/11- and Gα12/13-dependent signalling leads to vasoconstriction and proliferation of PASMC, whereas Gαs-dependent signalling, leads to vasodilation and decreased proliferation (Figure 1). Gαs-coupled GPCRs increases [cAMP], 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 Gαi, Gαq/11 and Gα12/13 tend to produce overlapping biological responses in PASMC, which leads to increased Ca2+ sensitisation and the phosphorylation of myosin light chain (MLC), promoting actin–myosin cross-bridging and PASMC contraction. Activation of Gαi-coupled GPCRs oppose the effects of Gαs-coupled GPCRs by decreasing [cAMP] by inhibiting adenylyl cyclases. Gαq/11-coupled GPCRs activate phospholipase C (PLC) leading to increased inositol-1,4,5-
triphosphate (IP₃) and [Ca²⁺]: and the phosphorylation of target proteins, such as Ca²⁺-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 Ca²⁺ sensitisation and sustained PASMC vasoconstriction can also result in stimulation of Gα₁₂/₁₃-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; Gα_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; Gα_s (11 GPCRs), Gα_i (8 GPCRs), Gα_q/₁₁ (16 GPCRs), and Gα₁₂/₁₃ (4 GPCRs). In parallel, in PAEC at least 18 GPCRs have been characterised, again with multiple coupling; Gα_s (2 GPCRs), Gα_i (5 GPCRs), Gα_q/₁₁ (14 GPCRs), and Gα₁₂/₁₃ (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 Gα_q/Gα₁₂/₁₃-coupled GPCRs in PAEC and Gα_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 PGI₂ via GPCRs on PAEC lead to vasoconstriction if they directly act on PASMC, via Gα/Gα_q/₁₁/Gα₁₂/₁₃ (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 \([\text{Ca}^{2+}]_i\) compared to control-PASMC \((\text{Zhang et al., 2007; Murray et al., 2011})\), 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 \((\text{Table 2})\). 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 \((\text{Lau et al., 2017})\). GPCRs whose altered expression or activity contribute to the imbalance of pulmonary vascular tone with PAH are outlined below: a \(\text{Ga}_i/\text{Ga}_q/\text{G}_{12/13}\) vs. \(\text{Ga}_s\) 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\(_{1-4}\), 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 A\(_2\) (TXA\(_2\)), respectively. IP, EP\(_2\), EP\(_4\) and DP\(_1\) are \(\text{Ga}_s\)-coupled and therefore increase cAMP and are vasodilatory in PASMC, whereas EP\(_1\), EP\(_3\), and FP and TXA are \(\text{Ga}_{q/11}\) or \(\text{Ga}_i\)-coupled so increase \([\text{Ca}^{2+}]_i\) or decrease cAMP and lead to vasoconstriction of PASMC \((\text{Hirata and Narumiya, 2011})\). 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 \((\text{Christman et al., 1992})\). Increasing PGI\(_2\) production by overexpressing PGI synthase in mice prevents the development of PAH \((\text{Geraci et al., 1999})\). 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 Gαs-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 Gαs-coupled) -and increased EP3 receptor expression (Gαi-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 EP1 receptors whose activation in PASMC would initiate vasoconstriction, whereas treprostinil has high affinity for DP1 and EP2 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 Gαs-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+}]; 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-tryptophan 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-HT_{1B} (Gα_i), 5-HT_{2A} (Gα_q), 5-HT_{2B} (Gα_q) and 5-HT_{7} (Gα_s) have shown to be expressed in the pulmonary circulation (Ullmer et al., 1995; Morecroft and MacLean, 1998): 5-HT_{1B} and 5-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-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-HT_{1B} receptor expression (5-HT_{1B}−/− mice) or activity (5-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 Gα_{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_{V}1.5 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 (AT₁, Gαq/11) and 2 (AT₂, Gαi); AT₁ expression is increased with PAH, whereas AT₂ is decreased (Morrell et al., 1999, De Man et al., 2012). AT₁ enhances the proliferation of PASMC via Gαq/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 AT₁ 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 AT₂ activation, due to their expression in PAEC, counteracts this proliferation by increased NO and prostacyclin production. AT₂ 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 AT₂ 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 AT₂ receptors can counter act the proliferative and vasoconstrictive of the ACE-ANG-II-AT₁ 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 AT₂ 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 AT1, has been identified to be an important 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 (Gαq- and Gαi-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 $\text{G}_i$/$\text{G}_q$/$\text{G}_{12/13}$ vs. $\text{G}_s$ 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 $\alpha_1$-adrenoreceptor (a$_1$-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; Molostov et al., 2008). Upregulation of these $\text{G}_q$/$\text{G}_{12/13}$/$\text{G}_i$-coupled receptors results in $[\text{Ca}^{2+}]_i$/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, S1P$_2$R 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 $\text{G}_i$/$\text{G}_q$/$\text{G}_{12/13}$ vs. $\text{G}_s$-dependent signalling, favouring vasoconstriction and proliferation of PASMC. Decreased $\text{G}_q$-coupled GPCRs in the endothelium and increased $\text{G}_i$/$\text{G}_q$/$\text{G}_{12/13}$-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: α1, β1 and β2-adrenoreceptors are decreased. Low-dose noradrenaline, via β1-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, P2Y₂, A₂A, LTB₄, 5-HT₂A 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 AT₁, ET₄ and P2Y₂, 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\(_{\text{v}}\)1.5 and 5-HT\(_2\)A in caveolae leads to 5-HT-dependent inhibition of K\(_{\text{v}}\) 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). Ga and by subunits dissociate upon receptor activation and initiate signalling. Additionally, recruitment of β-arrestins can also initiate G-protein independent signalling and trafficking. Ga5 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. Ga1i activation inhibits AC activity thereby reducing cAMP, which in turn leads to PASMC vasoconstriction and proliferation. Gaq activation promotes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) generating intracellular messengers 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG activates Protein Kinase C (PKC) while IP3 stimulates intracellular release of Ca2+ which then form a complex with a Ca2+ binding protein- calmodulin. Ga12/13 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) Ga1/Gaq/Ga12/13-coupled GPCRs that have been implicated in PASMC vasoconstriction and proliferation through. (Blue) Ga5-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


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


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


**G-protein dependent signalling**

- VPAC₂, IP, EP₃, EP₂, DP₁, β₂-AR, A₁₁, CGRP
- Gα₁
- cAMP (EPAC; PKA)

**G-protein independent signalling**

- ATP, ET₄, EP₂, FP, TP, α₁-AR, P2Y₁₂, PAR₁, PAR₂
- Gα₁/11
- Gα₁₂/13
- RhoGEF
- IP₃, DAG, Ca²⁺, PKC, ROCK

**Increased MLC phosphorylation dependent PASMC Vasoconstriction & Increased Proliferation**

**PASMC Vasodilation & Decreased Proliferation**

**Arrestin dependent signalling and internalisation**
GPCRs upregulated in PAH-PASMC

- ET_{A/B} - \( G_{a_5} \)
- VPAC-2 - \( G_{a_5} \)
- \( \alpha_1 \)-AR - \( G_{a_5/11} \)
- \( A_{T_1} \) - \( G_{a_5/11} \)
- EP_{3} - \( G_{a_5} \)
- 5-HT_{1B} - \( G_{a_5} \)
- 5-HT_{2A/3} - \( G_{a_5/12/13} \)
- SLP_{2} - \( G_{a_5/12/13} \)
- PAR_{1-3} - \( G_{a_5/12/13} \)
- CaS - \( G_{a_5/12/13} \)

GPCRs downregulated in PAH-PASMC

- IP - \( G_{a_5} \)
- AT_{2} - \( G_{a_i} \)
- DP1 - \( G_{a_s} \)
<table>
<thead>
<tr>
<th>Endogenous ligand</th>
<th>GPCR</th>
<th>Cell type</th>
<th>G-protein</th>
<th>Cell specific response</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT₁</td>
<td>PASMC</td>
<td>Gaq/11</td>
<td>Proliferation</td>
<td>Vasoconstriction/</td>
<td>(Yamada et al., 1996; Morrell et al., 1999)</td>
</tr>
<tr>
<td>AT₂</td>
<td>PAEC</td>
<td>Gαi</td>
<td>Vasodilation</td>
<td>anti-proliferation/</td>
<td>(Lee et al., 2010; Bruce et al., 2015)</td>
</tr>
<tr>
<td>ANG 1-7</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Shenoy et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Endothelin-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETₐ</td>
<td>PASMC</td>
<td>Gaq</td>
<td>Proliferation</td>
<td>Vasoconstriction/</td>
<td>(MacLean et al., 1994; McCulloch et al., 1998; Shichiri et al., 1997; Sakai et al., 2016)</td>
</tr>
<tr>
<td>ETₐ</td>
<td>PAEC</td>
<td>Gaq</td>
<td>Vasodilation</td>
<td>Anti-apoptosis</td>
<td></td>
</tr>
<tr>
<td>Noroepinephrine / Epinephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β₂-AR</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Boe and Simonsson, 1980; Leblais et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>α₁-AR</td>
<td>PASMC</td>
<td>Gαq/Ga12/13</td>
<td>Vasoconstriction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α₂-AR</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Norel et al., 1996)</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₁</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Norel et al., 1996)</td>
<td></td>
</tr>
<tr>
<td>M₃</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Norel et al., 1996)</td>
<td></td>
</tr>
<tr>
<td>M₃</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(Norel et al., 1996)</td>
<td></td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B₂</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>Apoptosis</td>
<td>(Taraseviciene-Stewart et al., 2005)</td>
</tr>
<tr>
<td>Vasopressin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Smith et al., 2006)</td>
<td></td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRL/RAM P</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(Upton et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VPAC-1</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(Busto et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>VPAC-2</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(Busto et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺, Mg²⁺, spermine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaS</td>
<td>PASMC</td>
<td>Gaq, Gα</td>
<td>Proliferation</td>
<td>Vasoconstriction</td>
<td>(Li et al., 2011)</td>
</tr>
<tr>
<td>Calcitonin gene-related peptide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGRP</td>
<td>PASMC</td>
<td>Gaq</td>
<td>Vasoconstriction/ anti-proliferation</td>
<td>(Chattergoon et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>Substance P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK₁</td>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Pedersen et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>NK₂</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(Pedersen et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₁</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(Ortiz et al., 1992)</td>
<td></td>
</tr>
<tr>
<td>Urotensin II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UT</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(MacLean et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₂A</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>Apoptosis</td>
<td>(Morgan et al., 1991; Xu et al., 2011; Huang et al., 2015)</td>
</tr>
<tr>
<td>A₂B</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(Morgan et al., 1991; Xu et al., 2011; Huang et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Oxytocin, Vasopressin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT</td>
<td>PASMC</td>
<td>Gαi, Gαq</td>
<td>Vasoconstriction</td>
<td>(Roberts et al., 1992; unpublished data)</td>
<td></td>
</tr>
<tr>
<td>ATP, ADP, UTP, UDP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P₂Y₂, P₂Y₄, P₂Y₆</td>
<td>PASMC</td>
<td>Gαq</td>
<td>Vasoconstriction</td>
<td>(McCormack et al., 1989; Chootip et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>PAEC</td>
<td>Gαq</td>
<td>Vasodilation</td>
<td>(McCormack et al., 1989; Chootip et al., 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td>5-HT1B</td>
<td>PASMC</td>
<td>Ga\textsubscript{i}</td>
<td>Proliferation/ Vasoconstriction/ Anti-apoptosis</td>
<td>(Morecroft and MacLean, 1998; Hoyer et al., 2002; Liu et al., 2013)</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------</td>
<td>-------</td>
<td>------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>5-HT2A</td>
<td>5-HT2A</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}/ Ga\textsubscript{12/13}</td>
<td>Proliferation/ Vasoconstriction/ Anti-apoptosis</td>
<td>(Morecroft and MacLean, 1998; Hoyer et al., 2002; Liu et al., 2013)</td>
</tr>
<tr>
<td>5-HT2B</td>
<td>5-HT2B</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Proliferation/ Vasoconstriction/ Anti-apoptosis</td>
<td>(Morecroft and MacLean, 1998; Hoyer et al., 2002; Liu et al., 2013)</td>
</tr>
<tr>
<td>Prostacyclin</td>
<td>IP</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Vasodilation/ anti-proliferation</td>
<td>(Shaul et al., 1991)</td>
</tr>
<tr>
<td>PGE\textsubscript{1/2}</td>
<td>EP\textsubscript{1}</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Vasoconstriction</td>
<td>Hirata and Narumiya, 2011</td>
</tr>
<tr>
<td></td>
<td>EP\textsubscript{2}</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Vasodilation</td>
<td>Hirata and Narumiya, 2011</td>
</tr>
<tr>
<td></td>
<td>EP\textsubscript{3}</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Vasoconstriction</td>
<td>Hirata and Narumiya, 2011</td>
</tr>
<tr>
<td></td>
<td>EP\textsubscript{4}</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Vasodilation</td>
<td>Hirata and Narumiya, 2011</td>
</tr>
<tr>
<td>Thromboxane</td>
<td>FP and TP</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}</td>
<td>Proliferation/ Vasoconstriction</td>
<td>(Cogolludo, 2003)</td>
</tr>
<tr>
<td>Sphingosine</td>
<td>SIP\textsubscript{1}</td>
<td>PAEC</td>
<td>Ga\textsubscript{i}</td>
<td>PAEC barrier protection</td>
<td>(Ancellin and Hla, 1999; Garcia et al., 2001; Birker-Robaczewska et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>SIP\textsubscript{2}</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}/ Ga\textsubscript{12/13}</td>
<td>Proliferation</td>
<td>(Cogolludo, 2003)</td>
</tr>
<tr>
<td></td>
<td>SIP\textsubscript{3}</td>
<td>PAEC</td>
<td>Ga\textsubscript{q}/ Ga\textsubscript{i}/ Ga\textsubscript{12/13}</td>
<td>PAEC barrier dysfunction</td>
<td>(Cogolludo, 2003)</td>
</tr>
<tr>
<td>Thrombin</td>
<td>PAR\textsubscript{1}</td>
<td>PAEC</td>
<td>Ga\textsubscript{q}/ Ga\textsubscript{12/13}</td>
<td>PAEC barrier dysfunction</td>
<td>(Sacks et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>PAR\textsubscript{1}/ PAR\textsubscript{2}</td>
<td>PASMC</td>
<td>Ga\textsubscript{q}/ Ga\textsubscript{12/13}</td>
<td>Proliferation/ Vasoconstriction</td>
<td>(Sacks et al., 2008)</td>
</tr>
<tr>
<td>Apelin; Elabela/Toddler</td>
<td>APJ</td>
<td>PAEC</td>
<td>Ga\textsubscript{q}/ Ga\textsubscript{i/o}</td>
<td>Vasodilation</td>
<td>(Japp et al., 2008; Yang et al., 2015)</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>NPY\textsubscript{1}</td>
<td>PASMC</td>
<td>Ga\textsubscript{i/o}</td>
<td>Proliferation/ Vasoconstriction</td>
<td>(Crnkovic et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>NPY\textsubscript{2}</td>
<td>PASMC</td>
<td>Ga\textsubscript{i/o}</td>
<td>Vasoconstriction</td>
<td>(Crnkovic et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>NPY\textsubscript{4}</td>
<td>PASMC</td>
<td>Ga\textsubscript{i/o}</td>
<td>Vasoconstriction</td>
<td>(Crnkovic et al., 2014)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>GPER</td>
<td>PAEC</td>
<td>Ga\textsubscript{i} / Ga\textsubscript{s}</td>
<td>Vasodilation</td>
<td>(Alencar et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PASMC</td>
<td>Ga\textsubscript{i} / Ga\textsubscript{s}</td>
<td>Vasodilation/ Anti-proliferative</td>
<td>(Alencar et al., 2017)</td>
</tr>
<tr>
<td>GPCR</td>
<td>Expression in PAH</td>
<td>Level of endogenous ligand</td>
<td>Drug</td>
<td>Effect</td>
<td>Refs</td>
</tr>
<tr>
<td>--------</td>
<td>------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td>-------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>ET&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>↑</td>
<td>↑</td>
<td>Bosentan</td>
<td>Antagonist (clinical)</td>
<td>(Rubin et al., 2002)</td>
</tr>
<tr>
<td>ET&lt;sub&gt;A&lt;/sub&gt;</td>
<td>↑</td>
<td>↑</td>
<td>Ambrisentan</td>
<td>Antagonist (clinical)</td>
<td>(Galie et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Macitentan</td>
<td>Antagonist (clinical)</td>
<td>(Pulido et al., 2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>*Sitaxsentan</td>
<td>Antagonist (clinical)</td>
<td>(Barst et al., 2004)</td>
</tr>
<tr>
<td>VPAC-2</td>
<td>↑</td>
<td>↓</td>
<td>Aviptadil</td>
<td>Agonist (clinical, in-vivo)</td>
<td>(Petkov et al., 2003; Leuchte et al., 2008)</td>
</tr>
<tr>
<td>IP</td>
<td>↓</td>
<td>↓</td>
<td>Epoprostenol</td>
<td>Analogue (clinical)</td>
<td>(Barst et al., 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Iloprost</td>
<td>Analogue (clinical)</td>
<td>(Olsheskiw et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treprostinil</td>
<td>Analogue (clinical)</td>
<td>(Simonneau et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beraprost</td>
<td>Analogue (clinical)</td>
<td>(Galiè et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Selexipag</td>
<td>Agonist (clinical)</td>
<td>(Simonneau et al., 2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MRE-269</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Fuchikami et al., 2017)</td>
</tr>
<tr>
<td>UT</td>
<td>-</td>
<td>-</td>
<td>Urantide</td>
<td>Antagonist (in-vitro)</td>
<td>(Mei et al., 2011)</td>
</tr>
<tr>
<td>CaS</td>
<td>↑</td>
<td>-</td>
<td>NPS 2143</td>
<td>NAM (in-vitro)</td>
<td>(Tang et al., 2016)</td>
</tr>
<tr>
<td>APJ</td>
<td>↓</td>
<td>↓</td>
<td>Apelin</td>
<td>Agonist (clinical)</td>
<td>(Brash et al., 2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Apelin/ ELA</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Yang et al., 2017)</td>
</tr>
<tr>
<td>A&lt;sub&gt;2B&lt;/sub&gt;</td>
<td></td>
<td>-</td>
<td>Adenosine</td>
<td>Agonist (clinical)</td>
<td>(Morgan et al., 1991; Rossi et al., 2017)</td>
</tr>
<tr>
<td>A&lt;sub&gt;2A&lt;/sub&gt;</td>
<td></td>
<td>-</td>
<td>LASSBio-1359</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Alencar et al., 2013)</td>
</tr>
<tr>
<td>AT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>↑</td>
<td></td>
<td>Losartan</td>
<td>Antagonist (in-vitro, in-vivo)</td>
<td>(Morrelli et al., 1999; De Man et al., 2012)</td>
</tr>
<tr>
<td>AT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>↓</td>
<td></td>
<td>C-21</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Bruce et al., 2015)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1B&lt;/sub&gt;</td>
<td>↑</td>
<td></td>
<td>GR127935</td>
<td>Antagonist (in-vitro, in-vivo)</td>
<td>(Keegan et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SB216641</td>
<td>Antagonist (in-vitro, in-vivo)</td>
<td>(Hood et al., 2017)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>↑</td>
<td></td>
<td>Ketanserin</td>
<td>Antagonist (in-vitro)</td>
<td>(McGoon and Vlietstra, 1987; Frishman et al., 1995)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2A/B&lt;/sub&gt;</td>
<td>↑</td>
<td></td>
<td>Terguride</td>
<td>Antagonist (in-vitro, in-vivo)</td>
<td>(Launay et al., 2002; Dumitrascu et al., 2011)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2B&lt;/sub&gt;</td>
<td>↑</td>
<td></td>
<td>PRX-08066</td>
<td>Antagonist (in-vitro, in-vivo)</td>
<td>(Porvasnik et al., 2010)</td>
</tr>
<tr>
<td>S1P&lt;sub&gt;2&lt;/sub&gt;</td>
<td>↑</td>
<td>↑</td>
<td>JTE013</td>
<td>Antagonist (in-vitro, in-vivo)</td>
<td>(Chen et al., 2014)</td>
</tr>
<tr>
<td>β&lt;sub&gt;2&lt;/sub&gt;-AR</td>
<td>-</td>
<td>-</td>
<td>Nebivolol</td>
<td></td>
<td>Agonist (in-vitro, in-vivo)</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>B9972</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Barst et al., 2004)</td>
</tr>
<tr>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>C1213</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Ahmed et al., 2016)</td>
</tr>
<tr>
<td>GPER</td>
<td>-</td>
<td>-</td>
<td>G1</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>(Alencar et al., 2017)</td>
</tr>
<tr>
<td>Mas</td>
<td>↓</td>
<td>-</td>
<td>ACE-2 and Ang 1-7</td>
<td>Agonist (in-vitro, in-vivo)</td>
<td>Agonist (in-vitro, in-vivo)</td>
</tr>
</tbody>
</table>

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