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Early View

Back to basics

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Neurohormonal modulation in pulmonary arterial hypertension

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Take home message

Better insight into neurohormonal changes throughout the development of pulmonary arterial hypertension and associated right heart failure is needed to allow efficacious intervention.

Abstract

Pulmonary hypertension is a fatal condition of elevated pulmonary pressures, complicated by right heart failure. Pulmonary hypertension appears in various forms; one of those is pulmonary arterial hypertension (PAH) and is particularly characterized by progressive remodelling and obstruction of the smaller pulmonary vessels. Neurohormonal imbalance in these patients is associated with worse prognosis and survival. In this back-to-basics review on neurohormonal modulation in PAH, we provide an overview of the pharmacological and non-pharmacological strategies that have been tested preclinically and clinically. The benefit of neurohormonal modulation strategies in PAH patients has been limited by lack of insight in how the neurohormonal system is changed throughout the disease and difficulties in translation from animal models to human trials. We propose that longitudinal and individual assessments of neurohormonal status are required to improve timing and specificity of neurohormonal modulation strategies. Ongoing developments in imaging techniques such as positron emission tomography (PET) may become helpful to determine neurohormonal status in PAH patients in different disease stages and optimize individual treatment responses.

Introduction

Pulmonary hypertension is a fatal condition of elevated pulmonary pressures, complicated by right ventricular failure. Pulmonary hypertension is classified into five groups. Group one is pulmonary arterial hypertension (PAH) and is characterized by progressive remodelling and obstruction of the smaller pulmonary vessels [1]. The resulting decrease in arterial diameter can increase blood pressure in the pulmonary circulation up to five times the normal pressure [2]. The pulmonary circulation then changes from a low- into a high-pressure circulation, placing an increased load on the thin-walled right ventricle (RV). This eventually leads to RV failure and death [3, 4]. There is currently no cure for PAH and treatment options are limited.

One of the disease modifiers in all forms of pulmonary hypertension, is neurohormonal imbalance, which has repeatedly been associated with poor clinical outcome and survival in patients [5–7]. Therefore, many pharmacological and non-pharmacological interventions on the neurohormonal system have been investigated in preclinical and few clinical studies [8]. Nevertheless, translation from preclinical to clinical studies is difficult and is often lacking.

Two recent reviews extensively described molecular pathways underlying the detrimental effects of neurohormonal activation [9] and pharmacological or invasive strategies targeting the neurohormonal

system in PAH [8]. We wish to extend on this topic, and thereby provide future directions for research into the neurohormonal system in PAH. In this back-to-basics article, we will therefore 1) recapitulate how the neurohormonal system ensures cardiovascular homeostasis; 2) describe how the neurohormonal system is changed in human PAH, both systemically and locally; 3) discuss how neurohormonal changes impact the progression of PAH and right heart failure; 4) give a comprehensive overview of the preclinical and clinical studies that intervene on the neurohormonal system in PAH.

Of note, neurohormonal changes likely play a role in all forms of pulmonary hypertension. Because most clinical data have been collected here, we focus in our review on PAH. Moreover, other forms of pulmonary hypertension are characterized by frequent co-morbidities (e.g. left heart failure or additional pulmonary diseases), complicating the view on the neurohormonal system. The neurohormonal changes and effects of interventions described hereafter are therefore focused on evidence obtained in PAH patients.

Back to basics

An important function of the neurohormonal system is to maintain cardiovascular homeostasis. Two pillars of the neurohormonal system are the autonomic nervous system (ANS) and the renin-angiotensin-aldosterone system (RAAS). The different components and functions of both systems are depicted in figure 1.

The ANS can be subdivided into two divisions with opposite actions: the sympathetic and parasympathetic nervous system (SNS and PNS, respectively), as shown in figure 1a. The SNS possesses adrenergic synapses and is generally activated under (physiological) stress to prepare the body for action, while the PNS has cholinergic synapses and prevails during rest. However, both systems are continuously active at basal levels and the net physiological effect depends on the balance between SNS and PNS activity.

The ANS ensures short-term control of cardiovascular homeostasis. Blood pressure and arterial CO_2 levels are continuously monitored by baro- and chemoreceptors in the aortic arch and carotid sinus. When arterial blood pressure is falling, baroreceptors are inactivated and baroreceptor-controlled inhibition of SNS is relieved. Meanwhile, PNS activity is suppressed, striking the balance towards the SNS. The SNS is also activated when rising CO_2 levels in the blood are being detected by chemoreceptors.

SNS activation then causes release of noradrenaline from synapses directly onto cardiac myocytes and blood vessels. In addition, sympathetic nerves stimulate synthesis and release from the adrenal gland of adrenaline and noradrenaline [10] that reach the heart and lungs via the bloodstream. (Nor)adrenaline then binds to the α- and β-adrenoreceptors (β-ARs). Increased signalling via ARs and reduced signalling from the parasympathetic nicotinic and muscarinic acetylcholine receptors (nAChR and mAChR) result in a net increase in the rate and force of myocardial contraction. In addition, stimulation of α-ARs that are highly expressed in the vasculature causes vasoconstriction, so that arterial pressure is restored.

Like the ANS, the RAAS is subdivided in two counteractive divisions: the classical and alternative RAAS system, as shown in figure 1b. The classical RAAS has been known for a long time and involves angiotensin converting enzyme (ACE), angiotensin (Ang) II, Ang II type 1 receptor (AT_1R), aldosterone and the mineralocorticoidreceptor (MR). The alternative RAAS system is less well-studied and involves ACE2, Ang1-7, Ang1-9 and the MAS receptor.

The RAAS ensures long-term control of blood pressure and volume. When arterial blood pressure drops, perfusion pressure of the kidney is reduced. As a consequence, renin is released from the kidney into the blood and facilitates the conversion of angiotensinogen to angiotensin I (Ang I). The latter is further converted to Ang II by ACE in the lungs [11]. Ang II acts as a strong vasoconstrictor via the AT_1R but can also increase the rate and force of myocardial contractility [12]. Furthermore, Ang II promotes aldosterone release from the adrenal glands via the MR to induce sodium and water retention in the kidneys and increase blood volume. However, aldosterone also directly acts on the heart, leading to cardiac hypertrophy and fibrosis [13]. These effects of aldosterone in the heart are independent from the increase in blood pressure. In the (pulmonary) vasculature, pathological levels of aldosterone are associated with oxidative stress and vascular inflammation [13].

In the opposing alternative RAAS pathway, Ang I is converted into Ang1-9 while Ang II is converted to Ang1-7, both by ACE2. Ang1-7 and Ang1-9 largely oppose the vascular effects of the classical RAAS pathway via the MAS receptor [14, 15]. Direct effects of Ang1-7 and Ang1-9 in the heart are unknown.

Neurohormonal systems interfere systemically and locally

The ANS and RAAS do not operate in isolation. Several interactions have been demonstrated, both centrally and locally, and mostly studied in chronic heart failure patients or experimental heart failure. Interactions at the system level include SNS-induced renin release from the kidneys [16, 17] and central

actions of Ang II in the brain. For example, it was shown that inhibition of classical RAAS signalling by ACE inhibitors or AT_1R blockers increases PNS activity [18] and reduces SNS activity [19]. The latter happens also when AT_1R blockade is applied to the brain only [20], suggesting that Ang II directly acts on the central nervous system. Furthermore, Ang II enhances chemoreceptor activation [21, 22] and inhibits baroreceptor reflex control of heart rate [20, 23], both leading to increased SNS activity. By contrast, SNS activity can be reduced by the alternative RAAS pathway via inhibition of noradrenaline release in the hypothalamus [24].

Interactions between the ANS and RAAS occur locally as well. For instance, Ang II increases adrenergic signalling by potentiating the release of noradrenaline from sympathetic nerve terminals [20] and inhibits cardiac noradrenaline re-uptake [25]. In addition, gene expression of the AT_1R in the heart is regulated by Ang II and the β_1AR [26, 27], suggesting that the balance of local SNS and classical RAAS determines gene expression of the AT_1R in the heart. Also in the pulmonary vasculature, cross-talk between β-AR and RAAS signalling has been described [6].

Systemic and local changes in the neurohormonal system in PAH patients

Systemic changes in the autonomic nervous system

PAH has repeatedly been associated with a systemic increase in SNS activity. SNS activity, directly measured via muscle sympathetic nerve activity (or MSNA) in a peripheral nerve, was increased in PAH patients [28, 29]. In addition, plasma levels of (nor)adrenaline were increased in PAH patients in most [30–33], but not all studies [34].

The SNS and PNS are connected in such a way, that increasing SNS activity will cause decreased PNS activity. PNS activity can be indirectly assessed by indices of heart rate variability and heart rate recovery after exercise. As expected, both heart rate variability indices for PNS activity [28, 35, 36] and heart rate recovery [37, 38] were found to be reduced in PAH patients. The autonomic imbalance towards SNS activity and away from PNS activity is related to a reduced exercise capacity [39, 40], worse New York Heart Association (NYHA) functional class [29, 41] and an increased mortality in PAH [30, 32].

Local changes in the autonomic nervous system

In PAH, the SNS is not only systemically activated, but also locally in the myocardium [33, 35, 42]. Important triggers for SNS activation are atrial and ventricular stretch: β-AR expression is downregulated

specifically in the right ventricle but not in the left ventricle in PAH patients [43], and was related to ventricular wall stress [44]. The role of atrial stretch on SNS activation was shown in studies investigating balloon atrial septostomy. Although, atrial septostomy was not designed to interfere with the neurohormonal system, it partially reduced muscle sympathetic nerve activity [45]. This reduction in muscle sympathetic nerve activity was related to the reduction in atrial pressure, suggesting that atrial stretch plays an additional role in SNS activation [45].

The acute increases in SNS are important to enhance the rate and force of cardiac contraction and thus to enable the RV to cope with the enhanced load. However, sustained cardiac SNS activation causes selective downregulation of β_1ARs and uncoupling of the remaining β-ARs from downstream signalling [43]. Reduced β-AR expression and signalling impairs RV function in at least two ways. First, although contractility (end-systolic elastance, Ees) and force generating capacity of RV cardiomyocytes are enhanced in PAH-patients [3], the loss of β-AR signalling reduces the RV contractile reserve [43, 46]. Loss of contractile reserve results in arterio-ventricular uncoupling and acute RV dilatation during exercise [47], and is associated with lower exercise capacity and reduced survival [48]. Second, reduced β-AR expression also plays an important role in impaired diastolic function in PAH. One of the downstream targets of the β-AR is protein kinase A (PKA), which regulates cardiomyocyte stiffness via phosphorylation of the giant sarcomeric protein titin [49]. Thus, reduced β-AR expression results in reduced PKA activity and PKA-mediated phosphorylation of titin, thereby increasing the stiffness of the RV cardiomyocytes [50]. In experimental PAH, sustained adrenergic activation has further been linked to cardiac hypertrophy, fibrosis, apoptosis and reduced capillary density [51, 52].

Sustained adrenergic activation is also implicated in pulmonary vascular remodelling. Impaired β-AR signalling causes loss of nitric oxide production by pulmonary artery (PA) endothelial cells , resulting in pulmonary vasoconstriction [6]. This is further enhanced by direct adrenergic stimulation of PA smooth muscle cells and collectively leads to chronic pulmonary vasoconstriction [6]. Moreover, continuous adrenergic stimulation causes PA smooth muscle cell hypertrophy and proliferation [53, 54].

As opposed to what is known about local changes in the SNS, changes in counteractive PNS signalling have been reported in the RV in only one study so far. Da Silva Gonçalves Bos *et al*. showed that nAChr expression is increased in the RV of PAH patients at end-stage, and that there is likely reduced degradation of acetylcholine by acetylcholinesterase in the synaptic cleft [36]. If, and how the PNS is changed specifically in the lungs is largely unknown.

Systemic changes in the Renin-Angiotensin-Aldosterone system

Imbalances in RAAS activation have been described in PAH as well. Plasma levels of Ang I and II are increased in PAH compared to healthy controls [55, 56]. However, Ang I and II levels are increased only in progressive PAH but not in stable PAH and are associated with disease progression and mortality [57]. Plasma aldosterone concentrations are higher in PAH patients than in controls with unexplained dyspnea but without PAH [58]. In a subgroup of treatment naïve patients from this study, aldosterone levels correlated positively with pulmonary vascular resistance and negatively with cardiac output [58]. However, in a larger cohort of PAH patients, aldosterone concentration was not directly associated with cardiac output, 6-minute walking distance (6MWD) or survival [59].

In addition to classical RAAS activation, alternative RAAS activation may be reduced in PAH. Decreased plasma levels of Ang 1-7 and 1-9 in patients were reported in one study [56], while unchanged Ang 1-7 levels were reported in another study [55]. In the latter study, a higher Ang II/Ang1-7 ratio indicated reduced conversion from Ang II to Ang 1-7 by ACE2 [55]. Plasma concentrations of ACE2 are not lower in PAH patients compared to controls, but it was suggested that auto-antibodies may reduce ACE2 activity [56]. Collectively, the balance within the RAAS seems to be in favour of classical RAAS activation, which is associated with worse disease progression and survival.

Local changes in the Renin-Angiotensin-Aldosterone system

In addition to systemic changes in classical RAAS signalling in PAH patients, local changes have been reported as well. The conversion from Ang I to Ang II by ACE happens predominantly in the lungs, where an overall decrease in pulmonary ACE activity was found [60]. This decrease however, may be partly because of a reduced endothelial surface area. By contrast, ACE activity was increased in isolated PA endothelial cells from PAH patients [57] and ACE expression was upregulated in smaller pulmonary vessels (intra-acinar arteries to capillaries) [61] and plexiform lesions [61, 62] of PAH patients. These findings implicate that the formation of Ang II is increased locally in the PAH pulmonary vasculature. In addition, distal pulmonary arteries from PAH patients have increased AT_1R expression [57]. Sustained Ang II exposure causes hypertrophy and proliferation of isolated patient PA smooth muscle cells via AT_1R signalling [57] and has been linked to vascular inflammation and fibrosis, and impaired endothelial function [12].

In addition, local aldosterone synthesis is possible in the pulmonary vasculature [63]. Changes in aldosterone levels or MR expression in the PAH lung have not been described. However, in experimental PAH and in isolated human PA smooth muscle cells and PA endothelial cells aldosterone has been associated with increased vascular remodelling [63–65].

Sustained classical RAAS activity has also unfavourable effects on the heart, including cardiomyocyte hypertrophy, fibrosis and conduction system disturbances [12]. The human heart possesses a local RAAS system, independent from but related to the circulating RAAS system [66]. Only one study was reported on local changes in RAAS activity in the heart in PAH. As opposed to the lungs, AT_1R expression was found to be decreased in the RV, despite increased ACE expression and Ang II formation [67]. Local changes in cardiac aldosterone signalling have not been described in PAH.

Little is known about local changes in alternative RAAS activity. With renewed interest in ACE2, the entry point for the SARS-CoV-2 virus, it was recently shown that mRNA of the soluble but not the membrane bound ACE2 is increased in explanted PAH lungs [68]. Although upregulated ACE2 can be considered beneficial in PAH, the implications of this shift towards soluble ACE2 for PAH patients are unknown. In the heart, ACE2 activity may be increased, as shown by increased formation of Ang1-7 from Ang II [69].

Neurohormonal modulation strategies in PAH

From the foregoing, it becomes clear that the neurohormonal system in PAH is out of balance both systemically and locally. Imbalances at both levels are associated with worse disease progression, survival or cardiac and vascular remodelling. Although increased SNS and classical RAAS activity are required to maintain cardiovascular homeostasis in case cardiac output drops, it is thought that chronic activation of SNS and RAAS eventually become maladaptive. This may lead to a vicious circle of further cardiac deterioration and increased neurohormonal balance, exacerbated by progressive pulmonary vascular remodelling. Therefore, the neurohormonal systems have been the target of several experimental treatments. Treatment strategies can be divided into pharmacological and non-pharmacological approaches. An overview of both treatment strategies on the ANS and RAAS is given in figure 2 and 3 respectively.

Table 1 - **The effect of pharmacological treatments targeted at different neurohormonal signalling pathways in preclinical and clinical studies.** ↑ Increased, = Unchanged, ↓ Decreased. SNS = sympathetic nervous system, PNS = parasympathetic nervous system, RAAS = renin-angiotensin-aldosterone system, mPAP = mean pulmonary artery pressure, PVR = pulmonary vascular resistance, ACE = angiotensin converting enzyme, AT_1 = Angiotensin II-receptor type 1, MR = mineralocorticoid receptor.

Target system	Treatment strategy	Preclinical	Clinical
SNS	β-blockers	Survival: ↑[70] Disease progression: ↓[71–73] Exercise capacity: ↑[51] Cardiac function: ↑[51, 73, 74], =[75] Cardiac remodelling: ↓[51, 76], =[75] Vascular remodelling: ↓[74, 77, 78], =[51]	Exercise capacity: =[79–81] Cardiac function: ↑[79], ↓[80]
PNS	Acetylcholinesterase inhibition	Disease progression: ↓[36] Cardiac function: ↑[36] Cardiac remodelling: ↓[36] Vascular remodelling: ↓[36]	
	Cholinergic agonists	Cardiac remodelling: ↓[82] Vascular remodelling: ↓[82]	
Classical RAAS	ACE inhibition	Disease progression: ↓[83, 84], Cardiac function: ↓[85], ↑[84] Cardiac remodelling: ↓[86–88], =[89], Vascular remodelling: ↓[83–90], =[89]	Exercise capacity: =[91–93] Cardiac function: =[92, 93] mPAP/PVR: =[91, 92] **(**mPAP&PVR**),** ↓[93] (mPAP)
	AT_1 blockers	Survival: ↑[94] Disease progression: ↓[57] Cardiac function: =[75] Cardiac remodelling: ↓[95–97], =[75, 94, 98] Vascular remodelling: ↓[57, 75, 96, 97], =[98, 99]	Exercise capacity: ↑[100], =[101] Quality of life: =[101] mPAP/PVR:↓[100](mPAP on echo)
	MR antagonists	Cardiac function: →[102–104*]	Exercise capacity: →[107, 108]

		Cardiac remodelling: ↓[63, 65, 103], →[102, 104*] Vascular remodelling: ↓[63, 65, 102, 103, 105, 106], →[65] *combined with losartan	Cardiac function: →[107] mPAP: →[107]
Alternative RAAS	ACE2 activation	Disease progression: ↓[109, 110] Cardiac function: ↑[109][110] Cardiac remodelling: ↓[111, 112][110] Vascular remodelling: ↓[109, 111–113][110]	Cardiac function: ↑[55]
	Ang1-7 administration	Cardiac function: ↑[110] Cardiac remodelling: ↓[110, 114], =[115] Vascular remodelling: ↓[110, 114], =[115]	
	Combined ACE2 and Ang1-7 administration	Cardiac function: ↑[110], =[116] Cardiac remodelling: ↓[116] [110] Vascular remodelling: ↓[116] [110]	
	Ang1-9 administration	Cardiac remodelling: ↓[14] Vascular remodelling: ↓[14]	

Pharmacological treatments

The effects of pharmacological treatments seizing on the different neurohormonal systems are summarized in Table 1. Below, we will discuss the few approaches that have been tested in patients.

β-blockers target the SNS and are the cornerstone of treatment of left ventricular failure, but current guidelines advice against the use of β-blockers in PAH [2]. A small pilot study demonstrated the safety of the non-selective β-blocker carvedilol [79] in patients. However, in a bigger randomized controlled trial RV function and 6MWD did not improve with carvedilol [81], despite lower resting heart rate and increased β-AR expression. To prevent possible peripheral vasodilation and blunted exercise-induced skeletal muscle blood flow, the β_1AR selective β-blocker bisoprolol was tested. In this study, a drop in cardiac index observed caused concern that cardiac function actually deteriorated [80]. Although no other indicators of progressive heart failure were seen, the lack of improvements in cardiac function and 6MWD gave no indication to prescribe bisoprolol to PAH patients. Genetic variation in the β_1AR can cause hypersensitivity to carvedilol but insensitivity to metoprolol and bisoprolol [117] and may cause different individual responses to β-blockers.

Pharmacological interventions on the classical RAAS include ACE inhibition, AT_1 blockade and MR antagonists. Clinical studies on ACE inhibition in pulmonary hypertension stem from the 1980s, when it was observed that captopril reduced both systemic- and pulmonary vascular resistance in congestive heart failure [118]. Two early studies indicated that captopril reduced systemic- but not pulmonary vascular resistance in PAH [91, 118]. By contrast, a significant decrease in pulmonary vascular resistance was found by Ikram *et al.* after only four days of captopril [93]. In this small study of five patients, the two youngest patients showed clinical improvements during three weeks of maintenance treatment. Collectively, these very small studies do not indicate a role for ACE inhibition by captopril in the treatment of PAH. In fact, the systemic vasodilatory effects of ACE inhibitors may exaggerate SNS and classical RAAS activities. While no further trials have been conducted, ACE inhibition may still have benefit in subgroups of patients.

AT_1 blockade has not been tested clinically in PAH. Instead, two clinical studies investigated the use of losartan in pulmonary hypertension (PH) secondary to chronic obstructive pulmonary disease (COPD) [101] and in PH secondary to lung disease or left ventricular failure [100]. Beneficial effects of losartan were observed after 8 weeks of treatment, including reduced mean pulmonary arterial pressure (mPAP) and increased exercise capacity [100]. However, in PH secondary to COPD losartan caused an early trend towards improvements in cardiac function and exercise capacity that was not

maintained throughout 12 months of treatment [101]. Thus, AT_1 blockade by losartan may have short-term but not long-term effects in PH patients.

Because of their diuretic action, MR antagonists are used to manage symptoms of RV failure in PAH. However, MR antagonists may have additional effects on the heart and lungs. Two studies investigated the use of the MR antagonist spironolactone in PAH. A retrospective analysis of patients using spironolactone while being enrolled in the ARIES-1 and -2 trial for the endothelin-receptor antagonist ambrisentan [119] revealed that spironolactone use enhanced the benefits of ambrisentan on 6MWD and the severity of PAH [108]. However, patients using spironolactone had generally more severe PAH at baseline. Therefore, in these patients the therapeutic potential of ambrisentan may have been higher, which would falsely suggest an effect of spironolactone. The use of spironolactone alone has recently been studied in 42 group I PAH patients, 19 of whom with idiopathic PAH [107]. Although no effects of spironolactone were found on markers of fibrosis, exercise capacity, disease progression or cardiac function, the use of spironolactone was safe and well-tolerated. An ongoing clinical trial (NCT01712620) aims to determine the clinical potential of spironolactone with longer treatment duration and earlier initiation.

Activating the counteractive alternative RAAS, instead of lowering classical RAAS activity, may also help to restore the balance within the RAAS. We are only beginning to recognize the role of the alternative RAAS system in the development of PAH. Therefore, to date only one clinical pilot study exists, investigating the effects of recombinant human ACE2 administration in five patients with either idiopathic PAH or hereditary PAH [55]. Importantly, no safety concerns were raised and ACE2 was well tolerated. In addition, short-term improvements in pulmonary haemodynamics and inflammatory status were observed, paving the way for additional trials into ACE2 administration.

Table 2 - **The effect of non-pharmacological treatments targeted at different neurohormonal signalling pathways in preclinical and clinical studies.** ↑ Increased, = Unchanged, ↓ Decreased. SNS = sympathetic nervous system, PNS = parasympathetic nervous system, RAAS = renin-angiotensin-aldosterone system, mPAP = mean pulmonary artery pressure, PVR = pulmonary vascular resistance

Target system	Treatment strategy	Preclinical	Clinical
SNS	Renal denervation	Survival: ↑[120] Disease progression: ↓[121] Cardiac function: ↑[121, 122] Cardiac remodelling: ↓[120–122] Vascular remodelling: ↓[120–122]	
	Pulmonary artery denervation	Disease progression: ↓[123] Exercise capacity: ↑[123] Cardiac function: =[124],↑[123, 125–127] Cardiac remodelling: ↓[123, 126, 127],=[124] Vascular remodelling: ↓[123, 127, 128],=[124]	Survival: =[129] Disease progression: ↓[129, 130] Exercise capacity: ↑[129–132] Cardiac function: ↑[129, 131, 132] mPAP/PVR: ↓[129–132]
	Transection of the cervical sympathetic trunk	Cardiac function: ↑[133] Cardiac remodelling: ↓[133] Vascular remodelling: ↓[133]	
PNS	Vagal nerve stimulation	Survival: ↑[52] Cardiac function: ↑[52] Cardiac remodelling: ↓[52] Vascular remodelling: ↓[52]	
Classical RAAS	N/A	N/A	
Alternative RAAS	N/A	N/A	

Non-pharmacological treatments

The results from preclinical and clinical studies into non-pharmacological strategies for neurohormonal modulation are summarized in Table 2. Only pulmonary artery denervation (PADN) has been studied in PAH patients.

Pulmonary artery baroreceptor activation reflexively causes pulmonary vasoconstriction [134, 135], probably via adrenergic nerves [136]. This provides the rationale for PADN as a way to reverse pulmonary vasoconstriction. Indeed, an acute drop in mPAP was observed in 13 PAH patients who underwent PADN [129]. This reduction in mPAP was maintained during three months of follow-up, even while patients were completely withdrawn from PAH medication. In addition, progressive improvement in haemodynamics, 6MWD and clinical status were shown 6 months post-PADN. These improvements were maintained after up to one year of follow-up [131]. The improvements in hemodynamic status and 6MWD were recently confirmed in a multi-centre open label trial, although the benefit in that study was less dramatic [130]. Importantly, Rothman *et al.* showed highly variable individual responses in patients who underwent PADN. While mPAP and pulmonary vascular resistance were reduced on average, in three patients the pulmonary vascular resistance actually increased [130]. Interestingly, however, no acute reductions in mPAP were observed in this study or in another case report [132], suggesting that mechanisms apart from relieving the reflexive vasoconstriction may play a role.

One such mechanism may be a general reduction in SNS activity. Stretch of the PA baroreceptors contributes to increased muscle sympathetic nerve activity in healthy individuals [137]. It is likely that this is the case in PAH patients too, given the extreme increase in PA pressure. In addition, PA baroreceptor stimulation increases the arterial baroreceptor setpoint and threshold [138], causing inactivation of arterial baroreceptors and thus increased SNS activity. However, heart rate at four or six months follow-up post PADN remained constant [130], suggesting that SNS activity was not reduced after the procedure.

Limitations for the use of neurohormonal modulation strategies in the clinic

So far, we have described systemic and local neurohormonal imbalance in PAH patients and strategies to restore them. Translational difficulties and lack of mechanistic insight in the development of neurohormonal imbalance in PAH patients have limited the use of neurohormonal modulation therapies.

Three quarters of all studies described in this review were performed in various animal models of PAH, mostly rat, mouse and pig. However, promising results from β-blockers or ACE inhibitors seen in animal studies have not been translated to human trials. Obviously, the usefulness of these animal models can be disputed. First, commonly used rodent models resemble PAH in some but not all aspects. Experimental PAH usually develops over the course of several weeks instead of years as is the case in human PAH. This potentially limits the development of compensatory mechanisms [139]. Second, clinical studies have always been conducted against a background of PAH-specific therapy, while animals received the study medication only.

Clinical studies into neurohormonal modulation in PAH come with their own limitations. Importantly, the therapeutic window for any treatment is small in end-stage PAH, and thus early intervention is usually advocated for [140]. However, it should be emphasized that neurohormonal changes are not solely maladaptive. In fact, acute changes in neurohormonal activity are vital in the maintenance of cardiovascular homeostasis and thus, countering the neurohormonal systems may at times be detrimental. At some point in the progression of PAH, the persistent neurohormonal activation becomes maladaptive. During this phase neurohormonal modulation may be beneficial. Longitudinal data on neurohormonal changes in either right- or left ventricular failure is scarce. In chronic congestive heart failure progressive increases in plasma levels of noradrenaline and renin have been described [141], suggesting progressive neurohormonal imbalance. In addition, levels of noradrenaline, Ang II and aldosterone that are found in failing hearts have been shown to exert deleterious effects on the heart and vasculature, as reviewed elsewhere [142], suggesting dose dependent effects of neurohormones. Thus, there seems to be an optimal treatment window to target the neurohormonal system, somewhere between the acute activation and chronic activation at pathologic levels. It is therefore pivotal to obtain longitudinal data of neurohormonal activity, in combination with hemodynamic and RV functional data.

Two important limitations have to be addressed that impede studies into neurohormonal changes over time. First, human tissue for investigation is hardly available and only from end-stage PAH. Therefore, it

is less suitable to study longitudinal changes. Second, PAH is a rare disease and most clinical studies were able to include a few dozens of patients at best. This makes it difficult to reach sufficient statistical power, let alone to test subgroups of patients or different drug dosages and timing. The low number of patients is an important difference with studies into neurohormonal modulation in LV failure, where neurohormonal modulation strategies have been implemented despite similar lack of insight into neurohormonal changes over time. This affects clinical studies in two ways: first, in LV failure large cohorts of patients can be retrospectively analysed to identify factors that contribute to better or worse efficacy of neurohormonal modulation strategies. This is impossible in PAH. Second, small clinical studies with broad inclusion criteria did hardly show beneficial effects of neurohormonal modulation in PAH so far. Failure to enrol large numbers of patients requires diligent patient selection to prove smaller, yet clinically significant treatment effects.

Both the availability of human tissue for investigation and the limited numbers of patients in clinical trials will not change. Longitudinal assessment of neurohormonal changes could tell when and where neurohormonal derailment becomes evident in the course of the disease, and thereby help to select patients for specific neurohormonal modulation strategies. In addition, it will tell whether neurohormonal modulation strategies are timely and targeted as they are intended. Therefore, we advocate for the development, validation and use of non- and minimally-invasive tools to monitor neurohormonal changes in PAH. In the following section, we will discuss possible tools, in different stages of development, that may help to improve our understanding of the neurohormonal changes in pulmonary hypertension.

Strategies for longitudinal assessment of neurohormonal changes in PAH

The gold standard to measure autonomic activity is to directly measure (para)sympathetic nerve trafficking by muscle microneurography, usually in a superficial skeletal muscle. Alternatively, determination of regional noradrenaline spillover allows for an organ-specific determination of SNS activation. Using microdialysis, local acetylcholine concentrations can be determined to measure PNS activity [143]. However, these measurements are all highly invasive and as such do not lend themselves for repeated testing. Instead, plasma (nor)adrenaline levels have been used to study SNS activation, but are not a reliable marker [29]. Alternatively, β-AR density in peripheral blood cells may serve as a marker of SNS (over-)activation [144]. Traditionally, determination of β-AR density relied on time consuming ligand binding assays. However, flow cytometric quantification of β-AR density was described recently

[145] allowing for easy, high-throughput testing. Using this technique, it was shown that β-AR density is lower in blood cells in PAH patients compared to healthy controls [146] and is dose-dependently increased by the β-blocker carvedilol [81]. The changes in peripheral blood cells thus seem to mimic the changes seen in the heart. Further studies are needed to validate the use of flow cytometric quantification of β-AR expression as a marker of SNS in PAH patients.

Measuring PNS activity via plasma acetylcholine concentrations is not feasible due to high rates of degradation and clearance in the synaptic cleft [143]. Instead, plasma acetylcholinesterase activity may potentially serve as a biomarker for decreased PNS activity [147] but requires validation in PAH patients.

Non-invasive indirect measures for PNS and SNS activities have been drawn from ECG parameters. There is natural variation in the time between consecutive heart beats, called heart rate variability. When expressed in the frequency-domain, high-, low- and very-low frequency (HF, LF, VLF respectively) spectral power can be distinguished [148]. The LF/HF ratio is commonly used to determine sympatho-vagal balance, assuming that LF power is generated by the SNS while HF power is generated by the PNS. However, this assumption does not hold true [148]. Not surprisingly therefore, most studies do not find correlations between the LF/HF ratio and direct measures of SNS and PNS activities [143]. This is especially true in PAH where an inverse relation between LF/HF ratio and muscle sympathetic nerve activity has been observed [28]. Furthermore, proper measurement and analysis of heart rate variability variables outside standardized laboratory settings is complicated [143]. Nevertheless, improved methods for derivation of heartbeat-derived autonomic measures have been described [149] and the emergence of wearable devices, such as smartwatches and activity trackers, allows for ambulatory recordings in realistic settings.

Another non-invasive way to determine specifically PNS function is to measure heart rate recovery after maximal exercise. Heart rate recovery was shown to be reduced in PAH patients, and was related to chronotropic incompetence and clinical worsening [36–38, 150, 151]. Heart rate recovery could easily and routinely be assessed by cardiopulmonary exercise testing (CPET). Even easier, however, would be the use of heart rate recovery after the 6-minute walking test, which is performed more frequently in the clinic. It was shown in heart failure patients, that the predictive value of heart rate recovery to predict survival is independent of whether the test is performed at maximal or sub-maximal intensity [152]. In PAH patients, it was shown that heart rate recovery after a 6-minute walking test was even more predictive for clinical worsening than the 6MWD itself [38]. However, a direct comparison for the

use of heart rate recovery from the 6-minute walking test or maximal CPET has neither been made in heart failure, nor in PAH patients. One study showed that PAH patients exhibit relatively higher aerobic capacity during the 6-minute walking test compared to CPET, despite lower heart rate, but did not compare the use of heart rate recovery as a marker for PNS activity [153]. It is worth investigating further whether these non-invasive measurements, potentially easily derived from ambulatory measurements or simple tests, could help to determine and monitor autonomic status in PAH patients.

Neurohormonal changes can also be studied using imaging techniques. Positron emission tomography (PET) may become an additional powerful tool to study neurohormonal activation in a clinical setting. Many PET tracers have now become available to study different aspects of the ANS, including presynaptic neurotransmitter recycling, β-AR density, PNS terminal nerves and mAChR expression [154].

PET tracers also exist to study RAAS activity, via ACE- and AT_1R expression [60, 155]. As ACE tracers usually accumulate in organs with high ACE expression they are especially suitable to visualize ACE activity in the lungs [155]. Less tissue specific, but much quicker and cheaper, is the assessment of plasma levels and activity of the several RAAS components. As already mentioned, plasma levels of Ang I and II are increased in progressive but not in stable PAH [57]. In addition, plasma renin levels are an independent predictor of mortality in PAH [156].

Collectively, ongoing developments in heart rate variability algorithms and imaging techniques hold promise for the measurement and separation of neurohormonal activities. Together with plasma levels and CPET, they may provide tools for longitudinal assessment of the neurohormonal system and to study the interrelation between local neurohormonal systems in different organs.

Conclusion

It is now clear that neurohormonal imbalance is involved in the development and progression of PAH. However, the neurohormonal system is vital for cardiovascular homeostasis. Thus, for successful clinical implementation, the timing and specificity of neurohormonal interventions needs to be improved. Because the number of patients available for clinical trials limit the use of subgroups, longitudinal assessment of neurohormonal activity is required. Non-invasive techniques such as imaging techniques may help to identify which patients may benefit from neurohormonal modulation, and at which phase of disease progression.

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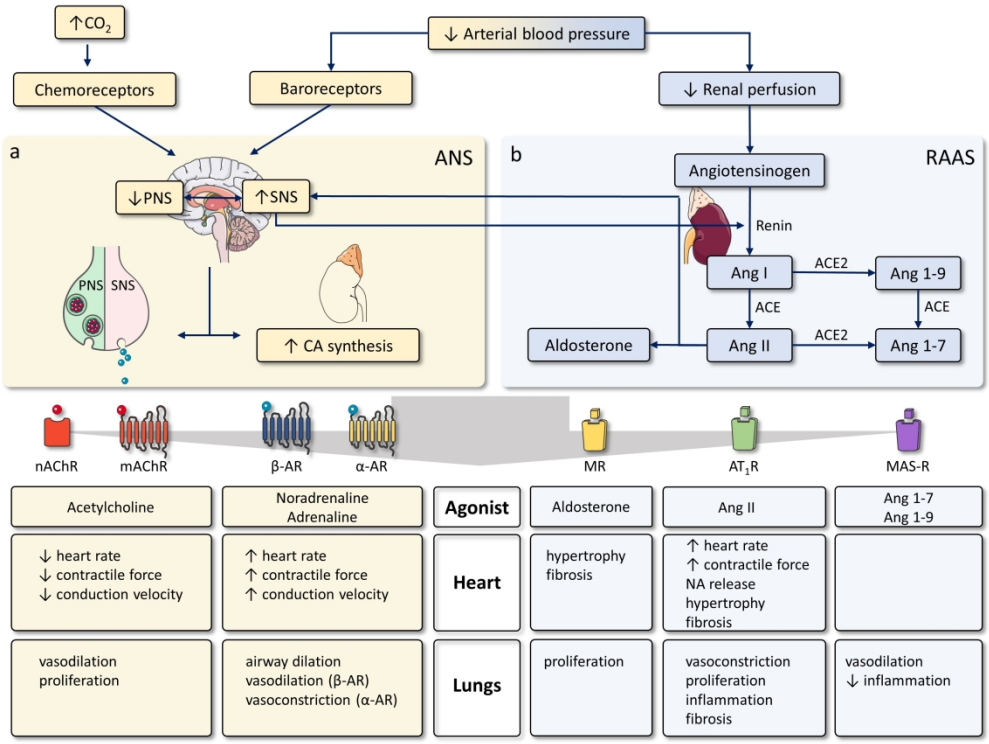

Figure 1. Short- and long-term control of blood pressure via neurohormonal signalling. PNS = parasympathetic nervous system, SNS = sympathetic nervous system, CA = catecholamine, ANS = autonomic nervous system, nAChR = nicotinic acetylcholine receptor, mAChR = muscarinic acetylcholine receptor, AR = adrenergic receptor, Ang = angiotensin, ACE = angiotensin converting enzyme, RAAS = renin-angiotensin aldosterone system, AT1R = angiotensin II receptor type I, MR = mineralocorticoid receptor.

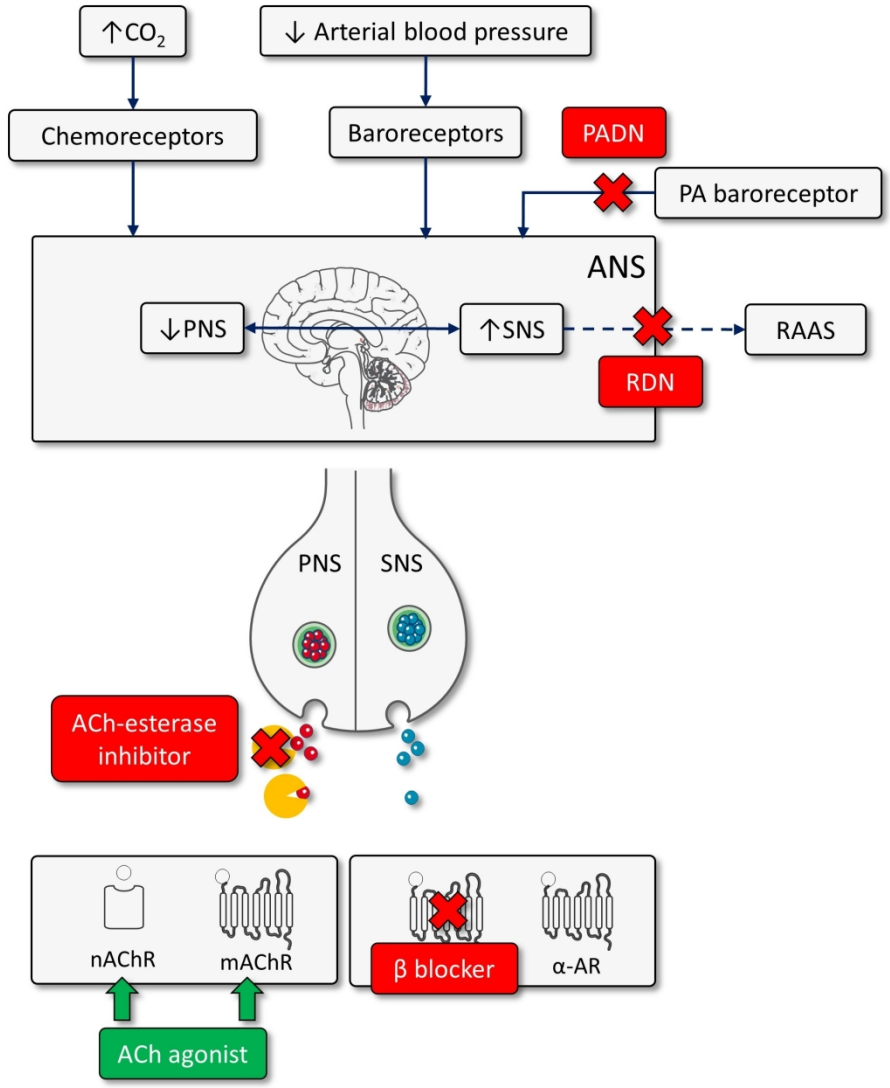

Figure 2. Targets of pharmacological and surgical interventions on the autonomic nervous system. PA = pulmonary artery, PADN = pulmonary artery denervation, PNS = parasympathetic nervous system, SNS = sympathetic nervous system, CA = catecholamine, ANS = autonomic nervous system, ACh = acetylcholine, nAChR = nicotinic acetylcholine receptor, mAChR = muscarinic acetylcholine receptor, AR = adrenergic receptor, RDN = renal denervation.

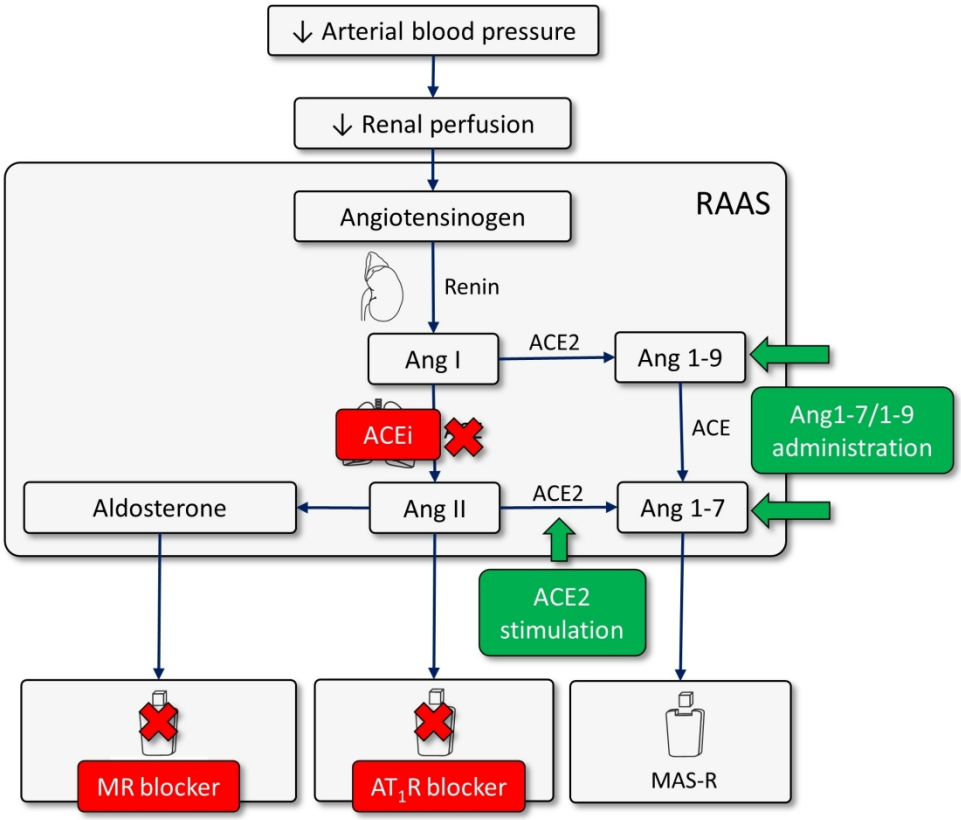

Figure 3. Targets of pharmacological and surgical interventions on the renin-angiotensin-aldosterone system. ACE = angiotensin converting enzyme, ACEi = angiotensin converting enzyme inhibitor, MR = mineralocorticoid receptor, AT1R = angiotensin II receptor type 1.