CHAPTER 6
Conclusions and further perspectives
Conclusions

In Figure 1 we can appreciate an overview of the findings of this thesis, and it demonstrates that increased neurohormonal activity, as increased sympathetic nervous system, renin angiotensin-aldosterone system activities and impaired parasympathetic activity may contribute to RV dysfunction and pulmonary vascular remodeling in PAH.

Figure 1: Neurohormonal dysfunction in PAH

By using a translational approach we demonstrated that local increase of sympathetic nervous system activity leads to downregulation of β-adrenergic receptor, reduced protein kinase A (PKA) mediated phosphorylation, impaired Ca²⁺ handling signaling, and it may contribute to increased RV diastolic stiffness in PAH. Despite the increase in sympathetic nervous system, increased renin angiotensin-aldosterone system (RAAS) may also contribute to increase RV stiffness and pulmonary vascular remodeling in PAH. Increased angiotensin II levels can bind to angiotensin II type 1-receptor (AT1) resulting in collagen production and deposition in the RV; and pulmonary artery smooth muscle cell proliferation and vasoconstriction in the pulmonary vasculature. In addition, increased aldosterone levels can contribute to RV fibrosis and pulmonary vascular remodeling, which could be mediated via mineralocorticoid receptor (MR) binding.
Besides the knowledge on the sympathetic nervous system and RAAS in PAH, less is known about the role of the parasympathetic nervous system. Previous studies have indicated that parasympathetic activity may be impaired in PAH-patients. However, its contribution to RV function and local RV cholinergic signaling is unknown. In our translational study we have demonstrated that reduced parasympathetic activity was associated with reduced RV function in PAH-patients. In addition, we reported for the first time that local cholinergic signaling may be impaired in RV from failing PAH-patients. We observed increased nicotinic receptor (α-7nAchR) expression, and reduced acetylcholinesterase activity in RV tissue from PAH-patients. These results suggest some local adaptation to impaired parasympathetic activity in PAH. However this adaptation may be insufficient to compensate the increase in sympathetic activity in PAH. The etiology of impaired parasympathetic activity in the context of PAH remains elusive and should be further investigated. Nevertheless, since sympathetic and parasympathetic nervous systems are linked to each other, it can be hypothesized that the impair in cholinergic signaling and on parasympathetic activity in PAH could be a compensatory mechanism due to increased sympathetic activity.

In addition to the role of neurohormonal dysfunction in PAH, we provided evidence that targeting the neurohormonal activity could be a promising therapeutic strategy for PAH. By using experimental animal models of PAH, we were able to demonstrate that suppression of RAAS by renal denervation resulted in reduction of RV diastolic stiffness and pulmonary vascular remodeling. Furthermore, parasympathetic nervous system stimulation with an acetylcholinesterase inhibitor, pyridostigmine, could be a potential target to improve RV function in PAH. Thus, by using an experimental PAH-rat model, we demonstrated that pyridostigmine treatment improved RV diastolic stiffness, pulmonary vascular remodeling, increased RV contractility and survival. Although we didn’t observe side effects with renal denervation and parasympathetic stimulation interventions, we should keep in mind that neurohormonal inhibitors may have some side effects, which could hamper its translation to clinical practice. Therefore further clinical studies should investigate the safety and efficacy of targeting the neurohormonal dysfunction in PAH-patients.

Exercise training is an adjunct therapy able to improve autonomic function in heart failure patients. However, less is known regarding the effect of exercise training on the cardiovascular autonomic function in PAH. Previously, we demonstrated that exercise training resulted in detrimental effects, as increased RV stiffness, in a progressive animal model of PAH. Hence, we further investigate whether local adrenergic and cholinergic signaling could explain this detrimental effect in the progressive PAH model. We were able to provide evidence that impaired RV: β-adrenergic receptor signaling, catecholamine reuptake and cholinergic response could be associated to the detrimental effect in progressive-PAH. These results suggest that autonomic function could be used to
distinguish the response to exercise training in PAH-patients. However, future clinical studies should investigate whether autonomic function could identify the PAH-patients who would benefit from exercise training.

Future Perspectives

Parasympathetic stimulation by pyridostigmine in patients with PAH
Encouraged by the promising results of parasympathetic stimulation in experimental PAH presented in Chapter 3, we would like to assess whether parasympathetic stimulation by the acetylcholinesterase inhibitor (pyridostigmine) would be also beneficial for PAH-patients. Therefore we will perform a proof-of-principle pilot study to investigate the safety and efficacy of pyridostigmine on the pulmonary vasculature and RV function of PAH-patients.

Role of alpha-7 nicotinic acetylcholine receptor on RV stiffness in PAH
In Chapter 3, we investigated the role of cholinergic signaling in RV from end-stage PAH-patients who underwent lung/heart transplantation. Previous study has demonstrated increased alpha-7 nicotinic acetylcholine receptor (α-7nAchR) expression in the left ventricle of a pressure-overload rat model. Accordingly, in Chapter 3, we demonstrated that RV tissue from PAH-patients also revealed higher α-7nAchR expression when compared to non-failing donors. Since, end-stage PAH samples were used for this study, it is difficult to distinguish if the increase on α-7nAchR expression is a compensatory mechanism due to autonomic dysfunction, or it also contributes to increase RV stiffness in PAH. Recently, Vang et al. revealed that cigarette smoke exposure in mice induces fibroblast proliferation and collagen content. They also provided evidence that these effects were mediated via α-7nAchR. Therefore, we hypothesize that the increase on α-7nAchR expression could contribute to RV stiffness in PAH. To solve the question whether this is a compensatory mechanism or it indeed results in RV stiffness, we will perform an experimental study using a knockout α-7nAchR mouse followed by PAH induction with pulmonary arterial banding (PAB). The PAB model is an isolated RV pressure overload model, which doesn’t take in account the pulmonary vascular remodeling. By inducing the PAH in knockout α-7nAchR and wild-type mice we can investigate the role of α-7nAchR in RV stiffness of PAH. If the increase in α-7nAchR contributes to RV stiffness, we will contribute to a novel potential therapeutic target for PAH.

Exercise training in PAH: which modality and for whom?
Exercise training is an adjunct therapy able to improve quality of life and exercise capacity of PAH-patients. In addition, exercise training is a promising adjunct therapy
to reduce sympathetic activity in patients with left heart failure.\textsuperscript{9, 10} However, less is known about the effect of exercise training on the cardiovascular autonomic function in the context of PAH. Moreover, it remains to be established whether exercise training is beneficial for all PAH-patients and which modality of exercise training would result in a better beneficial effect for PAH-patients.

Previous studies have demonstrated the beneficial effects of high intensity interval aerobic training in improving exercise capacity and left ventricle function in heart failure patients.\textsuperscript{18, 19} Recently, Brown \textit{et al.}\textsuperscript{20} have demonstrated that high intensity interval training was superior than continuous training in reverse the RV dysfunction in experimental PAH model. However it is not known which modality of exercise training will have superior beneficial effects in PAH-patients. For this reason, we will perform a randomized clinical trial to compare which exercise modality (continuous \textit{versus} interval training) will result in better improvements on exercise capacity, RV function and cardiac autonomic function in PAH-patients. Furthermore, we aim to investigate whether cardiac autonomic function could distinguish the patients who will respond to exercise training. Therefore, to investigate the overall cardiovascular autonomic function we will perform heart rate variability analysis before and after the exercise training program. In addition, the local changes on adrenergic and cholinergic signaling, will be assessed by PET scan analyses also before and after the exercise training protocol. The β-adrenergic receptor density and pre-synaptic norepinephrine will be assessed by using specific tracers, \textsuperscript{[11]}C-CGP-12177 and \textsuperscript{[11]}C-hydroxyephedrine.\textsuperscript{21} Furthermore, the cholinergic pre-synaptic acetylcholinesterase will be assessed by \textsuperscript{[11]}C-donepezil and the nicotinic receptor (α-7nAchR) by a novel tracer \textsuperscript{[11]}C-NS-14492.\textsuperscript{21, 22}
References


