Phosphodiesterase 1 as a future drug target in chronic heart disease

Det Medisinske Fakultet

Universitetet i Oslo

Gustav Bowitz Lothe

Våren 2017

Litteraturstudie ved det medisinske fakultet

Veiledere: Jan Magnus Aronsen og Professor Ivar Sjaastad, Institutt for Eksperimentell Medisinsk Forskning, Oslo Universitetssykehus Ullevål

Abstract

Background: Chronic heart disease imposes a significant disease burden to society. Of patients diagnosed with heart failure, 30-40% die within one year. Even with the advent of new treatments mortality decreased only by 10% absolute risk reduction between 1995 and 2005.

Objective: The aim of this project thesis is to explore a potential role for PDE1 as a mediator of cardiac pathology and as target for future pharmacological therapy of cardiac disease based on the available literature.

Method: A systematic literature searche in PubMed performed during Q1 2017. Eight search terms was applied in PubMed, and three predefined inclusion criteria was used to evaluate whether each article should be included for further analysis in this project thesis.

Results: The systematic literature search identified a total of 68 publications, and of these 20 were included based on the predefined inclusion criteria. Based on the included publications, the role of PDE1 as possible future treatment target was analyzed for cardiac hypertrophy and heart failure, pulmonary hypertension and arterial hypertension. For these three disease categories, experimental evidence from preclinical models was available. These were consistent with beneficial effects for PDE1 inhibition in cardiac hypertrophy, heart failure and pulmonary hypertension. The role of PDE1 inhibition in arterial hypertension was little studied, and little or no literature was available for the effect of PDE1 inhibition in other cardiac diseases.

Conclusion: Existing evidence hints at a potential for a future role of PDE1 inhibition in management of cardiac hypertrophy and cardiac failure as well as pulmonary hypertension.

Table of contents

Abbreviations

ADH	Anti-diuretic hormone
AMP	Adenosine monophosphate
BFBO	Berberis orthobotrys
Ca^{2+}	Calcium
CaMK	Ca2+/calmodulin-dependent protein kinase
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanine monophosphate
CVD	Cardiovascular disease
ECC	Excitation-contraction coupling
HDAC	Histone deactylase
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
LTCC	L-Type Calcium Channel
NCD	Non-communicable diseases
NCX	Sodium/calcium exchanger
NFAT	Nuclear factor of activated T cells
NO	Nitric oxide
PDE	Phosphodiesterase
PKA	Protein kinase A
PKG	Protein kinase G
RAAS	Renin-angiotensin-aldosterone system
RyR	Ryanodine receptor
SERCA2	SR calcium ATPase
SR	Sarcoplasmatic reticulum
TGF-ß	Transforming growth factor-ß

1. Introduction

1.1 What is chronic heart disease and how common is it?

Cardiovascular disease is an overarching term for diseases involving the heart or blood vessels. Cardiovascular disease (CVD) is the leading cause of mortality globally. In 2012, CVD caused 17.5 million deaths which constituted 46% of all deaths due to non-communicable diseases (NCD). In comparison, cancer, caused 8.2 million deaths which constituted 22% of all deaths due to NCD.(1) Improved treatment of hypertension and coronary disease are allowing patient to survive an early death. This survival, along with an aging population is leading to a sharp increase in heart failure. (2) Even with optimal treatment the median time of survival for men with congestive heart failure is only 1.7years and for women 3.2-years. (3) Clearly there is much room for improvement.

Heart failure occurs when the heart is unable to pump enough blood to meet the demands of the body or is able to do so only at an elevated filling pressure. Heart failure is usually referred to as heart failure with reduced ejection fraction (HFrEF) or with preserved ejection fraction (HFpEF). HFrEF is often referred to as systolic heart failure, and is characterized by reduced ability of the left ventricle to contract. In HFpEF (often referred to as diastolic heart failure) the ability of the left ventricle to relax is impaired.

One of the major contributing factors to disease in the cardiovascular system is hypertension. This can be systemic, or confined to the pulmonary circulation, also known as pulmonary hypertension. In order to maintain adequate perfusion of all organ systems the heart needs to overcome the vascular resistance. Chronic exposure to hypertension often leads to pathologic changes in cardiac tissue that is detrimental to the health of the patient.

1.2 The pathophysiology of chronic heart disease

In order to compensate for the inadequate circulation, the body activates several mechanisms in an attempt to improve the delivery of blood and oxygen. Activation of the sympathetic system, through baroreceptors, leads to an increase in catecholamines (adrenalin and noradrenalin) in an effort to maintain cardiac output with an increase in heart rate, increased myocardial contractility, and peripheral vasoconstriction. Chronic

sympathetic activation severely negatively impacts heart function over time. Excessive and chronic sympathetic activation is associated with cardiac myocyte apoptosis, hypertrophy and focal myocardial necrosis. (4)

Activation of the renin-angiotensin-aldosterone system (RAAS) also leads to vasoconstriction (angiotensin) and an increase in blood volume, through the retention of salt and water (aldosterone). The increase in blood volume will lead to an increase in contractility through the Frank-Starling principle. Furthermore, there may be progressive cardiac dilatation or alterations in cardiac structure (remodelling), or both. A third and important hormonal regulator of cardiovascular function are natriuretic peptides. The natriuretic peptides antagonize some of the effects of RAAS activation by inducing natriuresis and vasodilation. Recently, there has been increased interest in promoting these effects by inhibiting the breakdown of these natriuretic peptides through the use of neprilysin inhibitors. (5)

While some of these mechanisms are helpful in improving the cardiac output in the short term, they are a significant contributor to the pathological development and long term mortality. Current treatment focuses on inhibiting these high level mechanisms, they fail to address and prevent pathology in the cardiomyocytes. In order to treat and prevent the underlying causes of chronic heart disease it is necessary to understand the basic regulators of cardiac physiology and their role in the pathophysiology of cardiac disease.

1.3 Cardiac contractility and excitation-contraction coupling

The contraction of the cardiomyocyte happen through a process called excitation-contraction coupling (ECC). In ECC, a depolarization of the cardiomyocyte membrane causes an influx of calcium through the L-type calcium channel (LTCC). This influx of calcium (Ca^{2+}) activates ryanodine receptors (RyR) which releases a larger amount of Ca^{2+} from the sarcoplasmatic reticulum (SR). This leads to an increase in the cytosolic Ca^{2+} concentration (coined a Ca^{2+} transient), which leads to a binding of Ca^{2+} to troponin C which triggers a contraction. The contraction is terminated by pumping the Ca^{2+} out of the cytosol, mainly either back in to the SR through the cardiac SR Ca^{2+}ATPase (SERCA2) pump or out of the cell through the sodium/calcium exchanger (NCX). (6) At the termination of the ECC,

approximately 30% of the Ca^{2+} is pumped out of the cytosol through the NCX and approximately 70% is pumped back into the SR via the SERCA2.

1.4 Short term regulation of cardiac contractility

The two main ways the cardiac contractility is regulated in the short term is through either altering the duration or amplitude of the Ca^{2+} transient, or by altering the sensitivity of the myofilaments to Ca^{2+}. ß-adrenergic stimulation of the cardiomyocyte stimulates adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP). cAMP will then activate protein kinase A (PKA). PKA phosphorylates several proteins important to ECC, including RyR, phospholamban and the LTCC. The phosphorylation of phospholamban leads to an increase in the activity of the SERCA2 pump and thus an increase in the SR Ca^{2+} content. This will in turn lead to an increase in the Ca^{2+} released from the SR by the RyR and an increase in amplitude of the Ca^{2+} transient followed by increased contractility. This whole cascade of events is terminated in part by the degradation of cAMP by a class of enzymes known as phosphodiesterases (PDE), which converts cAMP into adenosine monophosphate (AMP). PDE's capable of decomposing cAMP include PDE 1, 2, 3, 4, 7, 8, 10, 11.

1.5 Impaired Ca^{2+} handling in the failing heart

In addition to being the signal responsible for cardiomyocyte contraction, Ca^{2+} serves several other roles as a second messenger (7). This functionality is dependent on maintaining a balanced homeostasis across the sarcolemma, cytosol and SR set by Ca^{2+} channels, ATP dependent transporters, and ion exchangers working in synergy with Ca^{2+} binding proteins. In heart failure, low SR Ca^{2+} is typically observed, which is thought to be a primary cause of contractile dysfunction as less Ca^{2+} is available for SR Ca^{2+} release in each contraction This decrease is mainly attributed to reduced activity by the SERCA2 pump and an increased leak of Ca^{2+} through the RyR channel. Studies have also shown an increased phosphorylation of the LTCC, leading to an increased influx of Ca^{2+} through the LTCC. Modification of this channel activity through pharmacological intervention has proven detrimental to survival in studies (8).

Another feature of the impaired Ca^{2+} handling in heart failure is the prolongation of the Ca^{2+} transient. This is primarily due to a decrease in SR Ca^{2+} uptake because of SERCA2a dysfunction. Studies in both animal models as well as human patients show a decreasd SERCA2a expression and activity. (9) This contributes to a decreasd SR Ca^{2+} content which in turn leads to a reduced Ca^{2+} release from the SR in diastole and contractile dysfunction.

1.6 Long term regulation of cardiac contractility and regulation of cardiac hypertrophy through second messenger's

In the presence of a chronic demand for an increased cardiac output, the body will attempt to increase the cardiac mass and contractile fibers in a process known as cardiac hypertrophy. Cardiac hypertrophy is a complex process that involves multiple signaling pathways and is still not fully understood. However, several signaling pathways have been identified and are associated with the development of cardiac hypertrophy. The hypertrophic response in highly trained athletes and in patients with pathologic cardiac disease seems to differ in several key ways (10). Here we will discuss pathologic cardiac hypertrophy.

Amongst the signaling pathways that mediate pathologic cardiac hypertrophy are the calcineurin–nuclear factor of activated T cells (NFAT), and endothelin induced increase in IP3. (11) These pathways are in part controlled by Ca^{2+} signaling. (12) Ca^{2+} also alters gene transcription through protein kinases (Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and CaMKIV) by altering the phosphorylation state of histone deactylases (HDACs).

1.7 Cyclic nucleotides and cardiac hypertrophy

Both cAMP/PKA and cyclic guanine monophosphate (cGMP)/ protein kinase G (PKG) signaling have been linked to cardiac hypertrophy. ß-adrenergic activation promotes cardiac hypertrophy and remodeling and have been shown to be mediated by cAMP/PKA activation(13). This effect is believed to be mediated by the global cAMP and PKA activity, but recent findings also indicates that local pools of cAMP have the opposite effect on cardiac hypertrophy, namely be preventing cardiac hypertrophy by phosphorylating NFAT (14). Thus, the hypertrophic effects of cAMP-PKA signaling might depend on the balance

between global and local cAMP levels, where the cAMP degrading enzyme phosphodiesterase 2 (PDE2) might provide an important barrier between these two compartments. PDE2 inhibition, in contrast to PDE3 and PDE4 inhibition, was able to reduce cardiac hypertrophy in a compartment that is suggested to reduce NFAT induced hypertrophy.

Other studies have also shown that cardiac hypertrophy can be prevented by activation of PKG by an unknown mechanism. cGMP is a second messenger protein that is produced by different guanylate cyclases that are activated by nitric-oxide (NO) and natriuretic-peptides. The termination of this signaling pathway is mediated through PDE's that decompose cGMP into guanylate monophosphate. PDE's capable of this decomposition include PDE 1, 2, 3, 5, 6, 9. It has been reported that PDE1 is the major cGMP hydrolyzer in the human myocardium. (15)

Phosphodiesterase 5 is a cGMP hydrolyzer capable of regulating NO generated cGMP. Numerous studies have shown the efficacy of PDE5 inhibition on preventing cardiac hypertrophy in animal models. (16) These same findings have however not been replicated in human trails. (17) Incidental findings have suggested that this may be due to different level of expression of PDE1 and PDE5 in human versus animal models.

In a 2015 study (18) Lee d coworkers were able to demonstrate the capacity for PDE9A inhibition to reverse pre-established heart disease. Phosphodiesterase 9 is a cGMP hydrolyzer capable of regulating natriuretic peptide generated cGMP in cardiomyocytes. These effects were shown to be PKG dependent. They were also able to demonstrate that the effects of PDE9 inhibition was PDE5 independent because PDE9 and PDE5 control different pools of cGMP. This was postulated to explain why PDE5 was unsuccessful in treating cardiomyopathy in some studies.

1.8 Phosphodiesterase 1

Phosphodiestarase 1 (PDE1), also known as Ca^{2+} -calmodulin dependent phosphodiesterase is an enzyme believed to play a pivotal role in the cross talk between cAMP, cGMP and Ca^{2+}

signaling. (19) PDE1 consists of three subtypes, PDE1A, PDE1B and PDE1C. Each subtype is further subdivided into various isoforms which are believed to be tissue specific. (20)

The different subtypes of PDE1 also exhibit different affinity for cAMP and cGMP. (21) PDE1A and PDE1B have both a higher affinity for cGMP then cAMP. A regulatory role for PDE1A and PDE1B on cGMP has been demonstrated in vivo in various cell lines. (22) PDE1C has almost equal affinity for both cAMP and cGMP with a slight advantage towards cAMP. However, it should be noted that despite similar affinity, the Vmax for cAMP is 10 times higher in PDE1C, and PDE1C has been showed to regulate cAMP in vivo (23).

Regulation of PDE1 is not fully understood, however, the activity of PDE1 is almost entirely dependent on the binding of Ca^{2+} and calmodulin. (20) Calmodulin is a small molecule which is able to bind two Ca^{2+} ions. There are two binding sites for calmodulin on the PDE1 enzyme. Thus, PDE1 will bind four Ca^{2+} ions. Inactivation of PDE1 is accomplished by phosphorylation by PKA or CaMKII on a single site, which will change the confirmation and greatly reduce the binding of calmodulin to the phosphodiesterase. (19) PDE1 is then dephosphorylated by Ca^{2+}-CaM-dependent protein phosphatase (calcineurin; protein phosphatase 2B), an event that happens in response to an increase in cytosolic Ca^{2+}. This suggests that PDE1 is inactivated by an increase in cAMP and is activated by an increase in intracellular Ca^{2+}.

The aim of this thesis is to explore a potential role for PDE1 as a mediator of cardiac pathology and as target for future pharmacological therapy of cardiac disease based on available literature.

2. Material and methods

In this paper, our aim has been to find relevant articles where PDE1 as a drug target can be connected to heart disease.

We performed a systematic search using these terms in https://www.ncbi.nlm.nih.gov/pubmed within the period Q1 2017:

Search terms

- PDE1 AND cardiomyopathy
- PDE1 AND cardiac hypertrophy
- PDE1 AND cardiac remodeling
- PDE1 AND hypertension
- PDE1 AND heart failure
- PDE1 AND aortic stenosis
- PDE1 AND arrhythmias
- PDE1 AND atrial fibrillation

Our criteria for inclusion were as following:

1. Original articles
2. Articles that investigate PDE1
3. Articles that investigate disease primarily within the cardiovascular system

Our criteria for exclusion were as following:

1. Reviews or Editorials
2. Articles primarily pertaining to other organ systems
3. Articles that do not investigate PDE1

3. Results

3.1 Number of results from pubmed by search term

The searches in Pubmed discovered a total of 68 articles tallied up from the different search terms with some overlap, i.e. that some articles were hits in more than one search. The total number of included papers was 20, which were distributed amongst the search terms as shown in Table 1. For a complete list of papers discovered in the search, see appendix 1.

Table 1

Search Term	**Number of results**	**Number included***	**References**
PDE1 AND cardiomyopathy	1	0	
PDE1 AND cardiac hypertrophy	7	5	(24) (25) (26) (27) (28)
PDE1 AND cardiac remodeling	8	3	(24) (29) (27)
PDE1 AND hypertension	32	14	(27) (30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (29) (25)
PDE1 AND heart failure	14	5	(24) (41) (25) (42) (43)
PDE1 AND aortic stenosis	1	1	(43)
PDE1 AND arrhythmias	5	1	(44)
PDE1 AND atrial fibrillation	0	0	
Total	**68**	**29 (20**)**	

*Several search terms have overlapping results. This is not indicated in this table.

** Number of unique articles included

3.2 Cardiac hypertrophy, fibrosis and heart failure

In this section, all identified papers that mainly investigated any role for PDE1 in mediating cardiac hypertrophy, fibrosis and/or heart failure will be collectively discussed. Whether PDE1 is regulated in any of the disease states will firstly be discussed collectively, then any reported functional effects by PDE1 in cells, before in vivo effects by PDE1 intervention is summarized in separate sections.

3.2.1 PDE1 expression and activity in cardiomyocytes

Several studies suggest that PDE1 signaling is upregulated in cardiac hypertrophy. Mokni and coworkers observed that PDE1A and PDE1C mRNA levels in left ventricles was increased after two weeks of angiotensin II treatment (25). This corresponded to their observation of increased cGMP PDE1 activity, but cAMP PDE1 activity was not measured. This is in line with the study by Yanaka and coworkers that observed PDE1A mRNA levels was slightly increased two weeks after aortic banding (28). This study also reported increased cGMP PDE1 activity in cardiac hypertrophy, which the authors of this study attributed to increased Ca^{2+}/calmodulin activation of PDE1. These studies are in correspondence to findings by Miller and coworkers that observed PDE1A expression to be upregulated in cardiomyocytes after TAC in mice, and after both ISO and angiotensin II treatment of cardiomyocytes (26).

Molecular changes in cardiac hypertrophy and heart failure compared to healthy individuals are often overlapping, but also many differences between the two disease states exist. In line with the observations of PDE1 regulation in hypertrophic hearts, Knight and coworkers observed upregulated PDE1 mRNA levels in transverse aortic constriction operated rats and also increased PDE1C protein levels in cardiac tissue from failing human hearts (24).

This corresponded to findings by Johnsen and coworkers, who determined that the contribution of PDE1 to total cAMP and cGMP PDE activity was surprisingly high (41). In this study more than 50% of the cAMP PDE activity was attributed to PDE1 and more than 80% of the cGMP PDE activity was attributed to PDE1 in humans. Corresponding findings of PDE1 activity in failing human hearts were reported by Vandeput and coworkers, who used sildenafil in various doses and demonstrated that sildenafil did not affect cGMP PDE activity

at concentrations specific to PDE5, but did significantly affect cGMP PDE activity at concentrations specific to PDE1 in failing human myocardium (42).

Altogether, there seems to be consistency in the literature that PDE1 is a significant hydrolyzer of cAMP and cGMP in the myocardium, and that PDE1 is upregulated both in hypertrophy and heart failure. Notably, PDE1 activity is high in failing human myocardium corresponding to the findings in preclinical models.

3.2.2 PDE1 expression and activity in cardiac fibroblasts

Cardiac fibrosis is a hallmark of chronic heart disease. It is in part caused by differentiation of cardiac fibroblasts into active, collagen secreting myofibroblast. Only two of the articles identified in this project thesis reported PDE1 expression and/or activity in cardiac fibroblasts (29) (24). The activated myofibroblasts in the study by Miller and coworkers showed an upregulation of PDE1A in animal cells.

This finding is in contrast to a study by Knight and coworkers who observed that PDE1C is upregulated in cardiomyocytes but not in cardiac fibroblasts (24).

3.2.3 Functional effects by PDE1 in cardiomyocytes and fibroblasts in vitro

The identified literature in this project thesis collectively reported three functional effects by PDE1 in cardiomyocytes and cardiac fibroblast in vitro. Namely, effects on hypertrophic, pathological growth of cardiomyocytes, apoptosis of cardiomyocytes and myofibroblast differentiation.

Cellular hypertrophy in vitro was induced by phenylepinephrine in a study by Miller and was evaluated by 3H-leucine incorporation, cell area size, and re-expression of the foetal gene program (26). Importantly, the presence of PDE1 inhibitor counteracted cellular hypertrophy in all assays in this work. This effect was linked to cGMP-PKG dependent effects as the authors of this study observed that inhibition of PDE1 lead to an increase in cGMP concentration and PKG activity in cardiomyocytes. Interestingly, another study using PDE1C deficient cardiomyocytes showed the anti-hypertrophic effects of PDE1C to be PKA dependent(24). Apoptosis in isolated cells was attenuated by PDE1 inhibition or PDE1C

inactivation after angiotensin II treated cardiomyocytes in a work by Knight and coworkers (24).This effects were reported to be PKA dependent.

Miller and coworkers studied the potential role for PDE1 in regulating the activity of myofibroblasts (29). PDE1 inhibition by the PDE1 selective inhibitor IC86340 or PDE1A shRNA significantly reduced Angiotensin II or transforming growth factor-ß (TGF-ß)-induced myofibroblast activation, collagen synthesis, and pro-fibrotic gene expression in rat myofibroblasts. Collectively, these three reports indicated PDE1 inhibition to exert anti-hypertrophic, anti-apoptotic and anti-fibrotic effects in vitro.

3.2.4 Cardiac effects of PDE1 inhibition in vivo

Of the studies included in this project thesis, three studies investigated whether PDE1 modulation in vivo had any disease modifying effects in preclinical disease models of cardiac hypertrophy, fibrosis and heart failure.

In rats that had ISO induced hypertrophy, Miller and coworkers observed reduced hypertrophy in animals treated with PDE1 inhibitor (26). This finding was confirmed ex vivo by the observation of less cell surface area, heart weight/tibia length and re-expression of the foetal gene program in animals treated with PDE1 inhibitor compared to control treatment. This data was thus in correspondence with anti-hypertrophic effects in single cells. Further, this finding was corroborated in another study using the same model, also by Miller and coworkers (29). This time, mice treated chronically with isoproterenol and PDE1 inhibitor was reported to have less fibrosis as evident by immunohistochemistry. In concert, these findings indicate that PDE1 inhibition to have both anti-hypertrophic and anti-fibrotic effects in mice chronically stimulated with ß-adrenergic agonists.

Importantly, and in correspondence to the findings in animal models with cardiac hypertrophy and fibrosis induced by ß-adrenergic activation, Knight and coworkers also found anti-hypertrophic and anti-fibrotic effects in PDE1C deficient mice after transverse aortic constriction. In addition, PDE1C deficient mice were resistant to heart failure development as they had preserved ejection fraction, preserved fractional shortening, and preserved left ventricular internal diameter compared to control animals (24). These in vivo

effects were confirmed by demonstration of reduced re-expression of the foetal gene program.

Altogether, three out of three studies that were included in this project thesis concluded that PDE1 inhibition in vivo exerted beneficial effects in two different models of chronic heart disease by demonstrating anti-hypertrophic, anti-fibrotic and cardio protective effects in mice.

3.3 Pulmonary Hypertension

In this section, all identified papers that mainly investigated any role for PDE1 in mediating pulmonary hypertension will be collectively discussed. Whether PDE1 is regulated in any of the disease states will firstly be discussed collectively, and any reported functional effects by PDE1 in cells before in vivo effects by PDE1 intervention is summarized in separate sections.

3.3.1 PDE1 expression and activity

In three of the studies included in this project thesis, PDE1 expression or activity was examined in lung arteries in patients or rodents with pulmonary hypertension. Two studies investigated PDE1 expression in human patients with pulmonary hypertension, Schermuly and coworkers demonstrated an upregulation of PDE1C mRNA in lung arteries in patients with idiopathic pulmonary artery hypertension (27). This finding was mirrored in a study by Murray and coworkers who also found that PDE1C mRNA and in addition PDE1A mRNA in lung arteries was strongly upregulated in both idiopathic pulmonary artery hypertension and secondary pulmonary artery hypertension (31).

Four studies investigated whether PDE1 activity was altered in pulmonary hypertension, two in rats and one in humans. The PDE activity was measured in rats exposed to chronic hypoxia by Maclean and coworkers (32). Here they show an increased PDE1 activity in lung arteries for cGMP and cAMP compared with controls. Phillips and coworkers were unable to demonstrate a significant contribution to cAMP PDE1 activity in healthy pulmonary artery smooth muscle cells and coronary artery muscle cells from rats (33).

In human samples, Murray and coworkers demonstrated a robust increase in cAMP PDE1 activity in both pulmonary artery smooth muscle cells from patients with either idiopathic pulmonary hypertension or secondary pulmonary artery hypertension (31). Altogether, these articles show an increased mRNA expression for PDE1A and PDE1C in pulmonary hypertension, and with one exception, PDE1 activity for cGMP and cAMP were robustly upregulated in lung arteries from idiopathic pulmonary hypertensive subjects. Importantly, all studies in human patients with pulmonary hypertension demonstrated upregulated PDE1 mRNA levels and activity.

3.3.2 Functional effects of PDE1 inhibition in pulmonary hypertension

Functional effects of PDE1 in pulmonary hypertension were examined in four papers that were included in this project thesis. One study examined the effect of PDE1 inhibitors in vascular smooth muscle cells, two papers investigated the effects of PDE1 inhibitors in isolated lungs and two studies examined the role of PDE1 in preclinical models of pulmonary hypertension.

Pulmonary hypertension is typically characterized by increased proliferation of vascular smooth muscle cells that contributes to the increased resistance to blood flow in this disease. Phillips and coworkers reported that PDE1 inhibitors induced less cell proliferation of vascular smooth muscle cells (33). In line with this, pulmonary artery pressure was reduced by PDE1 inhibition in isolated lung specimens from rats and mice with pulmonary hypertension (33). In the study by Schermuly and coworkers, PDE1 inhibition was shown to reduce vascular resistance in both healthy controls and mice with pulmonary hypertension, and increased sensitivity towards PDE1 inhibitor was observed in the disease animals (27). Thus, the functional data in isolated lungs are consistent with both PDE1 inhibition reducing vascular resistance in lung arteries, and increased PDE1 activity in this disease.

Of the three included studies that investigated PDE1 inhibitor modulated pulmonary hypertension, two were performed in models of long term remodeling after pulmonary hypertension and one in an acute model of pulmonary hypertension induced by thromoboxane. Schermuly and coworkers tested infusion of PDE1 inhibitor concomitantly with iloprost inhalation on rabbits and found a lower pulmonary artery blood pressure in the

treated animals compared to controls (30).This finding was later extended by Schermuly and coworkers (27). They observed reduced systolic pressure in the right ventricle, and less hypertrophy of the right ventricle after PDE1 inhibition in rats with both monocrotalin induced pulmonary hypertension and hypoxia induced pulmonary hypertension.

In a model of acute pulmonary hypertension in sheep, PDE1 inhibition did not alter pulmonary or systemic hemodynamics alone, but augmented the NO induced reduction in blood pressure (34).

Altogether, both studies that investigated PDE1 inhibitors in animal models with chronic pulmonary hypertension reported improved pulmonary hemodynamics by PDE1 inhibition, while the role of PDE1 inhibition in acute preclinical models were less clear. No reports on pulmonary hemodynamics in human patients were identified.

3.4 Hypertension

In this section, all identified papers pertaining to the role of PDE1 in hypertension will be discussed. In the first section, all results on PDE1 expression levels and activity in hypertension models are described, while functional effects by PDE1 in hypertension is discussed in the last section.

3.4.1 PDE1 expression and activity

Of the studies included in this project thesis, two studies investigated PDE1 expression in systemic arteries and one study investigated PDE1 activity in the aorta. Giachini and coworkers observed that in a hypertension model where rats was treated with angiotensin II, upregulated levels of protein for PDE1A and PDE1C in the aorta and small mesenteric arteries was measured (37). A similar observation was done by Dollé and coworkers who observed increased mRNA and protein levels of PDE1A and PDE1C in a mouse model of vascular aging (Ercc1d$^{-/-}$) compared to control mice (40).

Only one study identified in this project thesis examined PDE1 activity in systemic arteries, Hubert and coworkers reported no PDE1 dependent cAMP degradation in aortic specimens

with or without functional endothelium in neither sham or aorta banded rats (43). None of the identified studies in this project thesis examined PDE1 activity in systemic arteries from hypertensive models. Thus, there is some evidence that PDE1 isoforms are upregulated in systemic arteries in hypertension, but the PDE1 levels in healthy controls are low, and no PDE1 activity was observed in aortic preparations from rats without hypertension.

In addition to local mechanisms for vasoconstriction, studies have been conducted to ascertain the role for PDE1 in regulating the release of systemic mediators of blood pressure. Renin is one such systemic regulator. Ortiz-Capisano and coworkers were able to determine that PDE1 is a regulator of renin release and that PDE1 inhibition blocked the release of renin from juxtaglomerular cells (38). Western blotting revealed the isoform involved to be PDE1C.

3.4.2 Functional effects of PDE1 signaling

Of the identified studies in this project thesis, three examined functional effects of PDE1 inhibition in vascular samples from animal models with hypertension or vascular aging. In addition, one study by Alamgeer and coworkers isolated the ethyl acetate fraction of Berberis orthobotrys (BFBO), a root discovered in Pakistan, and tested its effects on isolated coronary artery rings (36). A significant relaxation was observed, however since BFBO was determined to affect multiple PDE's it is impossible to determine the contribution from PDE1 in this regard.

Thieme and coworkers did not observe any changes in either renal blood flow or blood pressure after PDE1 inhibition in mice that had been infused with angiotensin II to create an acute hypertension (35). Despite a small relaxant effect of PDE1 inhibition in aortic rings ex vivo, PDE1 inhibition also induced dilation of aortic rings from an animal model of vascular aging, where Ecc1d deficient mice had increased effect compared to controls.

Increased response to PDE1 inhibition in aorta rings was also reported by Giachini and coworkers, who observed that rats treated with angiotensin II for 14 days to induce hypertension had an increased vasoconstrictive response to phenylephrine, measured as

vascular contraction in the aorta and small mesenteric arteries, and that this effect could be abolished by PDE1 inhibition (37).

In total, three articles discuss the effects of specific PDE1 inhibition in vascular samples from animal models with hypertension or vascular aging. All of them were able to demonstrate an effect by PDE1 inhibition directly on the aorta, however only the study by Thieme and coworkers measure systemic blood pressure, and they did not observe any effects by PDE1 inhibition.

3.5 Other Cardiac Diseases

One outcome in this project thesis was that the results from the searches roles of PDE1 in arrhythmias, atrial fibrillation, myocardial infarction and cardiomyopathy, had a total of one article matching the inclusion criteria as described in Materials and methods and shown in Table 1 and Appendix 1. This indicates that little is known on the role of PDE1 in these diseases. In this section, the included papers within these search terms will be discussed collectively.

Kauman and coworkers examined the effects of PDE1 inhibition on basal sinoatrial rate and noradrenalin evoked tachycardia in right rat atria (44). At high concentrations, PDE1 inhibition caused marginal tachycardia but did not significantly change the chronotropic potency of noradrenaline. PDE1 inhibition did not alter the basal sinoatrial rate.

Other than that, this project thesis did not identify any other publications on PDE1 and cardiac electrophysiology and arrhythmias.

4. Discussion

In this project thesis we have performed a systematic literature search with the aim to evaluate a possible role for PDE1 as a future drug target in chronic cardiac disease. 20 original papers matched our inclusion criteria and the majority of papers had been investigating various roles of PDE1 in cardiac hypertrophy, fibrosis, heart failure and pulmonary hypertension. Altogether, PDE1 inhibition seems as a promising future target for treatment of these disease states based on results in rodent models of heart disease which will be discussed further in the following section.

One of the great challenges in any investigation of PDE1 function is the lack of isoform specific inhibitors. Other methods for teasing out the effects of the different isoforms exist and should be employed in order to gain a better understanding of the role of each isoform. This would greatly help in determining which pathway(s) is/are central in the cardio protective effects demonstrated and in developing future treatments that would utilize these pathways. Of the three isoforms of PDE1, both PDE1A and PDE1C have been shown to serve distinct roles in the myocardium. PDE1A have been implicated in regulating cardiomyocyte hypertrophy (26), while PDE1C have been implicated in the proliferation of smooth muscle cells of the aorta (23), and PDE1A was found to be upregulated in pulmonary smooth muscle cells (27). It should however be noted that as long as isoform specific PDE1 inhibitors are not available, both isoforms would have been inhibited in all instances and the upregulation that is discovered in all instances could be correlation and not causal. The development of transgenic mouse models with selective PDE1C inactivation is an important step forward to obtain more knowledge on isoform specific mechanism, but could be biased of other roles of PDE1 that cAMP/cGMP degradation such as protein-protein interactions with other proteins. Thus, any effect by either PDE1 inhibitors or PDE1 isoform inactivation should be interpreted with care.

4.1 Cardiac hypertrophy, fibrosis and heart failure

As described in the results section, several studies have investigated whether PDE1 is upregulated in cardiomyocytes and fibroblasts in vitro and in vivo after hypertrophic

remodeling. While PDE1A seems to be the dominant isoform regulating fibroblast activation, results in cardiomyocytes seems to be less clear. By using siRNA in isolated cardiomyocytes Miller and coworkers concluded that PDE1A was that main isoform inducing hypertrophy in vitro (26). A later study demonstrated clear cardioprotective effects by PDE1C inactivation in TAC operated mice (24). Thus, the role of PDE1A versus PDE1C in mediating cardiac hypertrophy is not clear, but both studies clearly indicate that PDE1 inhibition is beneficial by limiting cardiac hypertrophy, fibrosis and cardiac dysfunction. A difference between these studies was that Miller and coworkers investigated PDE1A inactivation in vitro after ß-adrenergic activation, while Knight and coworkers used TAC induced hypertrophy in PDE1C deficient mice in vivo. Another difference was that the PDE1A dependent effect was reported to be mediated through cGMP/PKG activity, while the PDE1C effect was due to cAMP-PKA activity. None of the studies reported downstream targets, and more studies are needed to investigate the separate roles of PDE1A/cGMP/PKG and PDE1C/cAMP/PKA pathways and their downstream targets in cardiac hypertrophy and remodeling. Little is known on the role of PDE1 in human chronic heart disease, but PDE1 activity measurements in failing human myocardium indicates high PDE1 activity as percentage of total PDE activity (41). Importantly there were no reports on negative effects of PDE1 inhibition in any of these studies, but notably, neither mortality, frequency of arrhythmias or side effects were reported in any of the included studies in this project thesis. Thus, PDE1 inhibition seems as a promising future treatment principle for cardiac hypertrophy, fibrosis and dysfunction, but more studies are needed before clinical testing is feasible. Among others to evaluate the risk for arrhythmias by PDE1 inhibition and to develop PDE1 inhibitors that is suitable for chronic administration in a clinical setting.

4.2 PDE5 versus PDE1 in clinical trials using sildenafil

No clinical trials with PDE1 inhibitors have been performed in human patients suffering from chronic heart disease, but trials using the PDE5 inhibitor sildenafil might shed some light on PDE1 and its possible cardioprotective role. An interesting property with sildenafil is its reported inhibitory effect also on PDE1. While sildenafil is generally regarded as highly selective for PDE5 inhibition, it is also known to be an inhibitor of PDE1 at higher concentrations. Several studies showing cardioprotective benefits in mice have been

conducted with concentrations of sildenafil ≥ 1.0 μM (16). At this concentration inhibition of both PDE5 and PDE1 is expected (26). The IC50 value for PDE1 is 350nM (45).

Human trails have already been carried out using the PDE5 selective inhibitor sildenafil. These trials did not show any cardioprotective effects in humans. Possible explanations for the treatment failure of PDE5 inhibition in humans could stem from the relative difference in the contribution to cGMP hydrolytic activity. In failing mouse hearts, the contribution of PDE5 accounts for 43% of the PDE1 activity of cGMP in left ventricular myocardium. Contrast this with studies in humans which have shown that PDE5 only contribute <5% of the PDE1 activity in failing hearts. (26)

This finding would suggest a role for PDE1 as a mediator of the cardioprotective effects shown in preclinical trials using sildenafil (46), Further credence to this possibility is gained from the article by (26) who do show a reduction in cardiac hypertrophy in vivo by PDE1 inhibition. Adding further evidence to this is the study by Knight et al (24) who also are able to demonstrate a reduction in myocyte apoptosis and hypertrophy, and demonstrated reduced fibrosis in animal models. In conclusion, no studies have directly targeted PDE1 in human trials, but results from trials using sildenafil for treatment of chronic heart provides another layer of evidence that indicates PDE1 to be cardioprotective in rodent models.

4.3 Pulmonary hypertension

Several studies included in this project thesis have investigated the role of PDE1 inhibition in pulmonary hypertension. Some notable observations highlights this as a promising future therapy for pulmonary hypertension. Firstly, PDE1 isoforms PDE1 and PDE1C seems to be heavily upregulated in human patients suffering from either idiopathic pulmonary hypertension or secondary pulmonary hypertension (31). Secondly, PDE1 inhibitors seems to reduce the systolic blood pressure in the lung circulation without having any significant systemic detrimental effects (27). This indicates that PDE1 mostly regulates arteries in the lung circulation and not in the systemic circulation. This is a clear advantage that will reduce the risk of side effects of PDE1 inhibitors if tested in patients with pulmonary hypertension. Thirdly, PDE1 inhibition was able to significantly reduce pulmonary blood pressure and also secondary right ventricular hypertrophy in the one preclinical trial in an animal model of

chronic hypertension (27). This was observed both for pulmonary hypertension induced by both hypoxia and monocrotaline. Little is known on underlying causes of idiopathic pulmonary hypertension in humans, and more studies on functional effects in lung tissue from these patient groups would probably facilitate later clinical trials to test PDE1 inhibitors against pulmonary hypertension. Nevertheless, PDE1 inhibition seems as an attractive strategy for treatment of pulmonary hypertension that is worth future investigations.

4.4 Hypertension

Investigations concerning hypertension as a possible target for PDE1 inhibition is still preliminary and more studies are needed as no preclinical trials of PDE1 inhibitors in animal models of arterial hypertension have been conducted. However, promising results indicate a mechanism for PDE1 in aging arteries as results have shown that senescent smooth muscle cells were able to regain most of their age induced loss of contractility with presence of a PDE1 inhibitor (40). Another possible benefit of targeting PDE1 in hypertension is the role that PDE1 plays in the releases of renin for the juxtaglomerular cells (38). As a major driver of blood pressure regulation, renin might contribute to hypertension. PDE1 inhibition seems to not alter blood pressure in healthy animals (35), and little is known to the effect of PDE1 inhibition in disease models of hypertension. One study used angiotensin II to induce chronic hypertension observed reduced blood pressure by PDE1 inhibition, which corresponded to other observations on upregulated PDE1 levels in arteries from hypertensive animals (37). However, more studies are needed in other animal models of hypertension, as for example spontaneously hypertensive rats or salt sensitive rats, to further verify if PDE1 is a potent target in hypertension.

5. Conclusion

Exciting evidence hints at a potential for a future role of PDE1 inhibition in management of cardiac hypertrophy and cardiac failure as well as pulmonary hypertension. Little is known on the role of PDE1 in other cardiac diseases and further studies are needed to evaluate this.

6. References

1. WHO | Mortality attributable to cardiovascular disease (CVD), cancer, diabetes or chronic respiratory disease (CRD) [Internet]. WHO. World Health Organization; [cited 2016 Sep 19]. Available from: http://www.who.int/gho/ncd/mortality_morbidity/ncd_four/en/

2. Epidemiology and causes of heart failure [Internet]. uptodate.com. [cited 2016 Sep 19]. Available from: http://www.uptodate.com/contents/epidemiology-and-causes-of-heart-failure?source=search_result&search=heart+failure+epidemiology&selectedTitle=1%7E150

3. Ho KKL, Pinsky JL, Kannel WB, Levy D, Pitt B. The epidemiology of heart failure: The Framingham Study. J Am Coll Cardiol. Journal of the American College of Cardiology; 1993 Oct 1;22(4s1):A6–A13.

4. Jackson G, Gibbs CR, Davies MK, Lip GY. ABC of heart failure. Pathophysiology. BMJ. BMJ Group; 2000 Jan 15;320(7228):167–70.

5. McMurray JJV, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin–Neprilysin Inhibition versus Enalapril in Heart Failure. N Engl J Med. Massachusetts Medical Society; 2014 Sep 11;371(11):993–1004.

6. Bers DM. Cardiac excitation-contraction coupling. Nature. Nature Publishing Group; 2002 Jan 10;415(6868):198–205.

7. Gorski PA, Ceholski DK, Hajjar RJ. Altered Myocardial Calcium Cycling and Energetics in Heart Failure—A Rational Approach for Disease Treatment. Cell Metabolism. Elsevier; 2015 Feb 3;21(2):183–94.

8. Mahé I, Chassany O, Grenard A-S, Caulin C, Bergmann J-F. Defining the Role of Calcium Channel Antagonists in Heart Failure Due to Systolic Dysfunction. Am J Cardiovasc Drugs. Springer International Publishing; 2003;3(1):33–41.

9. Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, et al. Relation between myocardial function and expression of sarcoplasmic reticulum Ca(2+)-ATPase in failing and nonfailing human myocardium. Circulation Research. American Heart Association, Inc; 1994 Sep 1;75(3):434–42.

10. Thienpont B, Aronsen JM, Robinson EL, Okkenhaug H, Loche E, Ferrini A, et al. The H3K9 dimethyltransferases EHMT1/2 protect against pathological cardiac hypertrophy. J Clin Invest. American Society for Clinical Investigation; 2017 Jan 3;127(1):335–48.

11. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. Nature Reviews Molecular Cell Biology. 2006 Aug;7(8):589–600.

12. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis

and remodelling. Nature Reviews Molecular Cell Biology. Nature Publishing Group; 2003 Jul 1;4(7):517–29.

13. Zhang X, Szeto C, Gao E, Tang M, Jin J, Fu Q, et al. Cardiotoxic and Cardioprotective Features of Chronic β-Adrenergic SignalingNovelty and Significance. Circulation Research. American Heart Association, Inc; 2013 Feb 1;112(3):498–509.

14. Zoccarato A, Surdo NC, Aronsen JM, Fields LA, Mancuso L, Dodoni G, et al. Cardiac Hypertrophy Is Inhibited by a Local Pool of cAMP Regulated by Phosphodiesterase 2. Circulation Research. Lippincott Williams & Wilkins; 2015 Sep 25;117(8):707–19.

15. Miller CL, Yan C. Targeting Cyclic Nucleotide Phosphodiesterase in the Heart: Therapeutic Implications. J of Cardiovasc Trans Res. Springer US; 2010;3(5):507–15.

16. Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, et al. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. Nat Med. 2005 Jan 23;11(2):214–22.

17. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, et al. Effect of Phosphodiesterase-5 Inhibition on Exercise Capacity and Clinical Status in Heart Failure With Preserved Ejection Fraction: A Randomized Clinical Trial. JAMA. American Medical Association; 2013 Mar 27;309(12):1268–77.

18. Lee DI, Zhu G, Sasaki T, Cho G-S, Hamdani N, Holewinski R, et al. Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease. Nature. Nature Publishing Group; 2015 Mar 26;519(7544):472–6.

19. Goraya TA, Cooper DMF. Ca2+-calmodulin-dependent phosphodiesterase (PDE1): Current perspectives. Cellular Signalling. 2005 Jul;17(7):789–97.

20. Kakkar R, Raju RVS, Sharma RK. Calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE1). Cell Mol Life Sci. Birkhäuser Verlag; 1999;55(8-9):1164–86.

21. Krieg T, Lukowski R, editors. Guanylate Cyclase and Cyclic GMP. Vol. 1020. Totowa, NJ: Humana Press; 2013.

22. Nagel DJ, Aizawa T, Jeon K-I, Liu W, Mohan A, Wei H, et al. Role of nuclear Ca2+/calmodulin-stimulated phosphodiesterase 1A in vascular smooth muscle cell growth and survival. Circulation Research. Lippincott Williams & Wilkins; 2006 Mar 31;98(6):777–84.

23. Rybalkin SD, Rybalkina I, Beavo JA, Bornfeldt KE. Cyclic nucleotide phosphodiesterase 1C promotes human arterial smooth muscle cell proliferation. Circulation Research. Lippincott Williams & Wilkins; 2002 Feb 8;90(2):151–7.

24. Knight WE, Chen S, Zhang Y, Oikawa M, Wu M, Zhou Q, et al. PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction. Proc Natl Acad Sci USA. National Acad Sciences; 2016 Oct 20;113(45):E7116–25.

25. Mokni W, Keravis T, Etienne-Selloum N, Walter A, Kane MO, Schini-Kerth VB, et al.

Concerted regulation of cGMP and cAMP phosphodiesterases in early cardiac hypertrophy induced by angiotensin II. Koch K-W, editor. PLoS ONE. Public Library of Science; 2010;5(12):e14227.

26. Miller CL, Oikawa M, Cai Y, Wojtovich AP, Nagel DJ, Xu X, et al. Role of Ca2+/calmodulin-stimulated cyclic nucleotide phosphodiesterase 1 in mediating cardiomyocyte hypertrophy. Circulation Research. Lippincott Williams & Wilkins; 2009 Nov 6;105(10):956–64.

27. Schermuly RT, Pullamsetti SS, Kwapiszewska G, Dumitrascu R, Tian X, Weissmann N, et al. Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy. Circulation. Lippincott Williams & Wilkins; 2007 May 1;115(17):2331–9.

28. YANAKA N, KUROSAWA Y, MINAMI K, KAWAI E, OMORI K. cGMP-Phosphodiesterase Activity Is Up-regulated in Response to Pressure Overload of Rat Ventricles. Bioscience, Biotechnology, and Biochemistry. Japan Society for Bioscience, Biotechnology, and Agrochemistry; 2014 May 22;67(5):973–9.

29. Miller CL, Cai Y, Oikawa M, Thomas T, Dostmann WR, Zaccolo M, et al. Cyclic nucleotide phosphodiesterase 1A: a key regulator of cardiac fibroblast activation and extracellular matrix remodeling in the heart. Basic Res Cardiol. Springer-Verlag; 2011 Oct 20;106(6):1023–39.

30. Schermuly RT, Inholte C, Ghofrani AH, Gall H, Weissmann N, Weidenbach A, et al. Lung vasodilatory response to inhaled iloprost in experimental pulmonary hypertension: amplification by different type phosphodiesterase inhibitors. Respiratory Research 2005 6:1. BioMed Central; 2005 Jul 20;6(1):76.

31. Murray F, Patel HH, Suda RYS, Zhang S, Thistlethwaite PA, Yuan JXJ, et al. Expression and activity of cAMP phosphodiesterase isoforms in pulmonary artery smooth muscle cells from patients with pulmonary hypertension: role for PDE1. AJP: Lung Cellular and Molecular Physiology. American Physiological Society; 2006 Aug 18;292(1):L294–L303.

32. Maclean MR, Johnston ED, Mcculloch KM, Pooley L, Houslay MD, Sweeney G. Phosphodiesterase Isoforms in the Pulmonary Arterial Circulation of the Rat: Changes in Pulmonary Hypertension. J Pharmacol Exp Ther. American Society for Pharmacology and Experimental Therapeutics; 1997 Nov 1;283(2):619–24.

33. Phillips PG, Long L, Wilkins MR, Morrell NW. cAMP phosphodiesterase inhibitors potentiate effects of prostacyclin analogs in hypoxic pulmonary vascular remodeling. AJP: Lung Cellular and Molecular Physiology. American Physiological Society; 2005 Jan 1;288(1):L103–15.

34. Evgenov OV, Busch CJ, Evgenov NV, Liu R, Petersen B, Falkowski GE, et al. Inhibition of phosphodiesterase 1 augments the pulmonary vasodilator response to inhaled nitric oxide in awake lambs with acute pulmonary hypertension. AJP: Lung Cellular and Molecular Physiology. American Physiological Society; 2006 Apr 1;290(4):L723–9.

35. Thieme M, Sivritas SH, Mergia E, Potthoff SA, Yang G, Hering L, et al. Phosphodiesterase 5 inhibition ameliorates angiotensin II-dependent hypertension and renal vascular dysfunction. Am J Physiol Renal Physiol. 2017 Jan 4;:ajprenal.00376.2016.

36. Alamgeer, Chabert P, Akhtar MS, Jabeen Q, Delecolle J, Heintz D, et al. Endothelium-independent vasorelaxant effect of a Berberis orthobotrys root extract via inhibition of phosphodiesterases in the porcine coronary artery. Phytomedicine. 2016 Jul;23(8):793–9.

37. Giachini FR, Lima VV, Carneiro FS, Tostes RC, Webb RC. Decreased cGMP Level Contributes to Increased Contraction in Arteries From Hypertensive Rats. Hypertension. American Heart Association Journals; 2011 Mar 1;57(3):655–63.

38. Ortiz-Capisano MC, Liao TD, Ortiz PA, Beierwaltes WH. Calcium-dependent phosphodiesterase 1C inhibits renin release from isolated juxtaglomerular cells. AJP: Regulatory, Integrative and Comparative Physiology. American Physiological Society; 2009 Oct 30;297(5):R1469–76.

39. Nose T, Kondo M, Shimizu M, Hamura H, Yamaguchi Y, Sekine T, et al. Pharmacological Profile of GPD-1116, an Inhibitor of Phosphodiesterase 4. Biological and Pharmaceutical Bulletin. The Pharmaceutical Society of Japan; 2016 May 1;39(5):689–98.

40. Dollé MT, Kuiper R, Roodbergen M, Robinson J, de Vlugt S, Wijnhoven SP, et al. Broad segmental progeroid changes in short-lived Ercc1−/Δ7mice. Pathobiology of Aging & Age-related Diseases. Portland Press Limited; 2017 Jan 18;1(1):7219–1075.

41. Johnson WB, Katugampola S, Able S, Napier C, Harding SE. Profiling of cAMP and cGMP phosphodiesterases in isolated ventricular cardiomyocytes from human hearts: Comparison with rat and guinea pig. Life Sciences. 2012 Feb;90(9-10):328–36.

42. Vandeput F, Krall J, Ockaili R, Salloum FN, Florio V, Corbin JD, et al. cGMP-Hydrolytic Activity and Its Inhibition by Sildenafil in Normal and Failing Human and Mouse Myocardium. J Pharmacol Exp Ther. American Society for Pharmacology and Experimental Therapeutics; 2009 Aug 20;330(3):884–91.

43. Hubert F, Belacel-Ouari M, Manoury B, Zhai K, Domergue-Dupont V, Mateo P, et al. Alteration of vascular reactivity in heart failure: role of phosphodiesterases 3 and 4. Br J Pharmacol. 2014 Dec 1;171(23):5361–75.

44. Kaumann AJ, Galindo-Tovar A, Escudero E, Vargas ML. Phosphodiesterases do not limit β1-adrenoceptor-mediated sinoatrial tachycardia: evidence with PDE3 and PDE4 in rabbits and PDE1–5 in rats. Naunyn-Schmied Arch Pharmacol. Springer-Verlag; 2009 Aug 20;380(5):421–30.

45. Bender AT, Beavo JA. Cyclic Nucleotide Phosphodiesterases: Molecular Regulation to Clinical Use. Pharmacol Rev. American Society for Pharmacology and Experimental Therapeutics; 2006 Sep 1;58(3):488–520.

46. Movsesian MA, Kukreja RC. Phosphodiesterase Inhibition in Heart Failure. In: Phosphodiesterases as Drug Targets. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. pp. 237–49. (Handbook of Experimental Pharmacology; vol. 204).

Appendix 1

Table 2

PDE1 AND cardiac hypertrophy	Lead author	Type of article	Included	Remarks
[Cyclic nucleotide phosphodiesterases: role in the heart and therapeutic perspectives].	Bedioune I	Review	NO	French
PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction.	Knight WE	Original	YES	
Cyclic nucleotide phosphodiesterases in heart and vessels: A therapeutic perspective.	Bobin P	Review	NO	
Concerted regulation of cGMP and cAMP phosphodiesterases in early cardiac hypertrophy induced by angiotensin II.	Mokni W	Original	YES	
Role of Ca2+/calmodulin-stimulated cyclic nucleotide phosphodiesterase 1 in mediating cardiomyocyte hypertrophy.	Miller CL	Original	YES	
Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy.	Schermuly RT	Original	YES	
Myocardial phosphodiesterases and regulation of cardiac contractility in health and cardiac disease.	Osadchii OE	Review	NO	
cGMP-phosphodiesterase activity is up-regulated in response to pressure overload of rat ventricles.	Yanaka N	Original	YES	
PDE1 AND cardiac remodeling				
PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction.	Knight WE	Original	YES	
Cardiac Phosphodiesterases and Their Modulation for Treating Heart Disease.	Kim GE	Review	NO	
Nanodomain Regulation of Cardiac Cyclic Nucleotide Signaling by Phosphodiesterases.	Kokkonen K	Review	NO	

Cyclic nucleotide phosphodiesterase 1A: a key regulator of cardiac fibroblast activation and extracellular matrix remodeling in the heart.	Miller CL	Original	YES	
Role of phosphodiesterases in adult-onset pulmonary arterial hypertension.	Murray F	Review	NO	
Targeting cyclic nucleotide phosphodiesterase in the heart: therapeutic implications.	Miller CL	Review	NO	
[Novel concepts in the pathobiology of pulmonary arterial hypertension].	Rosenkranz S.	Review	NO	German
Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy.	Schermuly RT	Original	YES	
PDE1 AND hypertension				
Phosphodiesterase 5 inhibition ameliorates angiotensin II-dependent hypertension and renal vascular dysfunction.	Thieme M	Original	YES	
[Cyclic nucleotide phosphodiesterases: role in the heart and therapeutic perspectives].	Bedioune I	Review	NO	
Endothelium-independent vasorelaxant effect of a Berberis orthobotrys root extract via inhibition of phosphodiesterases in the porcine coronary artery.	Alamgeer	Original	YES	
Cyclic nucleotide phosphodiesterases in heart and vessels: A therapeutic perspective.	Bobin P	Review	NO	
Pharmacological Profile of GPD-1116, an Inhibitor of Phosphodiesterase 4.	Nose T	Original	YES	
Role of Phosphodiesterase 5 and Cyclic GMP in Hypertension.	Mergia E	Review	NO	
Phosphodiesterase 1 regulation is a key mechanism in vascular aging.	Bautista Niño PK	Original	YES	
Cyclic nucleotide phosphodiesterase 1 and vascular aging.	Yan C.	Editorial	NO	
Modulation of Polycystic Kidney Disease Severity by Phosphodiesterase 1 and 3 Subfamilies.	Ye H	Original	NO	Does not pertain to relevant organ

				system or disease.
Phosphodiesterase 1A modulates cystogenesis in zebrafish.	Sussman CR	Original	NO	Does not pertain to relevant organ system or disease.
NADPH oxidase 4 deficiency reduces aquaporin-2 mRNA expression in cultured renal collecting duct principal cells via increased PDE3 and PDE4 activity.	Féraille E	Original	NO	Does not pertain to relevant organ system or disease.
Vasodilatory activity and antihypertensive profile mediated by inhibition of phosphodiesterase type 1 induced by a novel sulfonamide compound.	Pontes LB	Original	NO	Study lacks sufficient evidence for the effect of the novel compound LASSBio-985 on PDE1
Cyclic nucleotide phosphodiesterase 1A: a key regulator of cardiac fibroblast activation and extracellular matrix remodeling in the heart.	Miller CL	Original	YES	
PDE1 isozymes, key regulators of pathological vascular remodeling.	Chan S	Review	NO	
Role of phosphodiesterases in adult-onset pulmonary arterial hypertension.	Murray F	Review	NO	
Decreased cGMP level contributes to increased contraction in arteries from hypertensive rats: role of phosphodiesterase 1.	Giachini FR	Original	YES	
Concerted regulation of cGMP and cAMP phosphodiesterases in early cardiac hypertrophy induced by angiotensin II.	Mokni W	Original	YES	
Expression and function of phosphodiesterases in nitrofen-induced congenital diaphragmatic hernia in rats.	van der Horst IW	Original	NO	Does not pertain to

				relevant organ system or disease.
Cyclic nucleotide signaling in polycystic kidney disease.	Wang X	Original	NO	Does not pertain to relevant organ system or disease.
Calcium-dependent phosphodiesterase 1C inhibits renin release from isolated juxtaglomerular cells.	Ortiz-Capisano MC	Original	YES	
Phosphodiesterase inhibition in heart failure.	Movsesian M	Review	NO	
[Novel concepts in the pathobiology of pulmonary arterial hypertension].	Rosenkranz S	Review	NO	German
Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy.	Schermuly RT	Original	YES	
Expression and activity of cAMP phosphodiesterase isoforms in pulmonary artery smooth muscle cells from patients with pulmonary hypertension: role for PDE1.	Murray F	Original	YES	
Phosphodiesterase type 4 expression and anti-proliferative effects in human pulmonary artery smooth muscle cells.	Growcott EJ	Original	NO	Wrong PDE
Inhibition of phosphodiesterase 1 augments the pulmonary vasodilator response to inhaled nitric oxide in awake lambs with acute pulmonary hypertension.	Evgenov OV	Original	YES	
Lung vasodilatory response to inhaled iloprost in experimental pulmonary hypertension: amplification by different type phosphodiesterase inhibitors.	Schermuly RT	Original	YES	
cAMP phosphodiesterase inhibitors potentiate effects of prostacyclin analogs in hypoxic pulmonary vascular remodeling.	Phillips PG	Original	YES	
4-(3-Chloro-4-methoxybenzyl)aminophthalazines: synthesis and inhibitory activity toward phosphodiesterase 5.	Watanabe N	Original	NO	Wrong PDE

Phosphodiesterase isoforms in the pulmonary arterial circulation of the rat: changes in pulmonary hypertension.	Maclean MR	Original	YES	
Antiplatelet and antiproliferative effects of SCH 51866, a novel type 1 and type 5 phosphodiesterase inhibitor.	Vemulapalli S	Original	NO	Study is nonspesific as to which PDE it targets and what effects can be attributed to each.
PDE1 AND heart failure				
[Cyclic nucleotide phosphodiesterases: role in the heart and therapeutic perspectives].	Bedioune I	Review	NO	
PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction.	Knight WE	Original	YES	
Cardiac Phosphodiesterases and Their Modulation for Treating Heart Disease.	Kim GE	Review	NO	
Cyclic nucleotide phosphodiesterases in heart and vessels: A therapeutic perspective.	Bobin P	Reveiw	NO	
Alteration of vascular reactivity in heart failure: role of phosphodiesterases 3 and 4.	Hubert F	Original	NO	Wrong PDE
[Role of cyclic nucleotide phosphodiesterases in the cAMP compartmentation in cardiac cells].	Mika D	Reveiw	NO	French
Profiling of cAMP and cGMP phosphodiesterases in isolated ventricular cardiomyocytes from human hearts: comparison with rat and guinea pig.	Johnson WB	Original	YES	
Phosphodiesterase inhibition in heart failure.	Movsesian MA	Reveiw	NO	
Concerted regulation of cGMP and cAMP phosphodiesterases in early cardiac hypertrophy induced by angiotensin II.	Mokni W	Original	YES	Appears in previous search

cGMP-hydrolytic activity and its inhibition by sildenafil in normal and failing human and mouse myocardium.	Vandeput F	Original	YES	
Phosphodiesterase inhibition in heart failure.	Movsesian M	Reveiw	NO	
[Novel concepts in the pathobiology of pulmonary arterial hypertension].	Rosenkranz S	Reveiw	NO	
Phosphodiesterase regulation of nitric oxide signaling.	Kass DA	Reveiw	NO	
Myocardial phosphodiesterases and regulation of cardiac contractility in health and cardiac disease.	Osadchii OE	Reveiw	NO	
PDE1 AND myocardial infarction				
Angiotensin-converting enzyme inhibition prevents myocardial infarction-induced increase in renal cortical cGMP and cAMP phosphodiesterase activities	Clauss F	Original	NO	Wrong organ system
Cyclic nucleotide phosphodiesterase 1A: a key regulator of cardiac fibroblast activation and extracellular matrix remodeling in the heart	Miller CL	Original	YES	
PDE1 AND arrhythmias				
Regulation of murine cardiac function by phosphodiesterases type 3 and 4.	Beca S	Review	NO	
Phosphodiesterases reduce spontaneous sinoatrial beating but not the 'fight or flight' tachycardia elicited by agonists through Gs-protein-coupled receptors.	Kaumann AJ	Review	NO	
Phosphodiesterases do not limit beta1-adrenoceptor-mediated sinoatrial tachycardia: evidence with PDE3 and PDE4 in rabbits and PDE1-5 in rats.	Kaumann AJ	Original	YES	
Potency, selectivity, and consequences of nonselectivity of PDE inhibition.	Bischoff E	Review	NO	
Pharmacological induction of delayed and prolonged cardiac protection: The role of prostanoids.	Szekeres L	Review	NO	

PDE1 AND cardiomyopathy				
[Cyclic nucleotide phosphodiesterases: role in the heart and therapeutic perspectives].	Bedioune I	Review	NO	French
PDE1 AND aortic stenosis				
Alteration of vascular reactivity in heart failure: role of phosphodiesterases 3 and 4.	Hubert F	Original	YES	
PDE1 AND atrial fibrillation				
No results				