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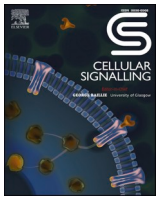

Phosphodiesterase-1 in the cardiovascular system

Anton J.M. Roks

Division of Vascular Pharmacology and Disease, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

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ABSTRACT

Phosphodiesterases regulate levels of the cyclic nucleotides cyclic adenosine monophosphate and cyclic guanosine monophosphate. Thanks to regulation by specific phosphodiesterase subtypes and isoforms in differential intracellular nanodomains, these versatile, ubiquitous signaling molecules can exert specific effects. This regulation depends on cell type and the (patho)physiological conditions in which these cells reside. In this review phosphodiesterase 1 is highlighted with respect to its structure, function and exploitation as a drug target for modulation of cardiovascular function. The function of its various isoforms in vascular smooth muscle, cardiac myocytes and fibroblasts are discussed. This comprises vasomotor control, cardiac myocyte contractility, growth control, fibrosis and senescence. The conditions that modulate phosphodiesterase 1 and the clinical relevance of this modulation are summarized. These conditions include proliferative status, cell stress, and aging. Furthermore, important associated signaling mechanisms and the implication of nanodomains are described. Also, the prospective of using PDE1 inhibitors as clinical drugs in cardiovascular disease is addressed.

1. Introduction

The cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are amongst the most important cellular messengers. Through their respective specific target protein kinase, protein kinase A (PKA) and protein kinase G (PKG), and through cyclic nucleotide-gated channels (NGC), they exert a plethora of functions such as cytoskeleton rearrangement, receptor and ion channel modulation and regulation of gene transcription through cAMP response element binding protein (CREB). Next to PKA, cAMP uses an alternative route, namely exchange proteins directly activated by cAMP, better known as EPACs [1]. In striated muscle of the heart, cAMP can also exert effects through Popeye domain-containing (POPDC) proteins, a family which is important for cardiac pacemaking and conduction [2]. The cAMP and cGMP is produced by adenylyl cyclases (AC) and guanylyl cyclases (GC) respectively [3]. To balance the signals prompted by increase of cAMP and cGMP in diverse regions within the cell, their local subcellular concentration is regulated by phosphodiesterases (PDEs) that convert the nucleotides to their non-cyclic form [4,5].

To enable a high level of signal specificity a large diversity of PDEs exists [5]. The superfamily of PDEs contains 21 different genes that together produce 11 subtypes (PDE1-PDE11). A number of subtypes form families that contain various isoforms (eg. PDE1A, B, C), and the various isoforms may be subdivided in various splice variants (eg. PDE1A1, 1A2) [6]. All together over 100 PDE variants have been identified [7]. Structurally, the PDE's all contain a catalytic domain, determining their affinity for each of the cyclic nucleotides, in the C-terminal half of the protein. The N-terminal half contains regulatory domains that determine enzymatic activity. For example, PDE subtypes of which the activity is regulated by cGMP,such as PDE3 and 5, contain a GAF domain, which is named after three earlier identified protein groups that contain similar cyclic nucleotide binding domains: c**G**MP-specific phosphodiesterases, *Anabaena* **a**denylyl cyclases and *Escherichia coli* **F**hlA). The PDE1–3 and PDE10–11 families are dual-substrate PDEs and are able to hydrolyze both cyclic adenosine monophosphate (cAMP) and cGMP. PDE4, −7 and − 8 are cAMP-specific and PDE5, −6, and − 9 are cGMP-specific. The enzymes exhibit a unique tissue distribution which is family- and isoform-dependent. This implies that PDEs are drug targets that may be suitable for pharmacotherapy that is relatively disease- and organ-specific [8].

In recent years subtype-specific inhibitors have been developed. Currently, PDE3, 4 and 5 inhibitors are applied clinically. PDE3 inhibitors strongly stimulate cardiac contraction, promote vasodilation and inhibit platelet aggregation, and are indicated for short-term use in acute heart failure, cardiogenic shock, and intermittent claudication [9]. In addition, they might be interesting for repurposing in cognitive disorders due to their effect on cAMP-related effects in memory control [10]. PDE4 inhibitors have been clinically registered for inflammatory disease, most prominently chronic obstructive pulmonary disease, asthma, and skin disorders. Possibly, PDE4 inhibition might also be

E-mail address: a.roks@erasmusmc.nl.

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effective in inflammatory disease of bowel, joints and neuronal tissue. The latter is relevant for potential application in neurological disorders such as Alzheimer's Disease [11,12].

PDE5 inhibitors are currently prescribed for reduction of pulmonary hypertension, because they inhibit proliferation and fibrosis in vascular smooth muscle cells (VSMC) and in fibroblasts, and for improvement of erectile dysfunction, as based on their effect on cGMP-induced relaxation in VSMC [3,13]. Potential application in cardiac, renal metabolic disease and non-atherosclerotic vascular aging is currently being investigated [14,15].

The clinically applied PDE inhibitors are to a certain degree selective for subtype, but are not isoform-specific. Isoform-specificity might further improve disease-specificity and reduce side effects. In the case of PDE3, which has two isoforms 3A and 3B, with 3 variants of 3A, isomer-specificity of the inhibitor might evade the increased mortality due to sudden death that is observed during chronic use of the current non-specific inhibitors [9]. In the case of PDE4, which has 4 isomers, 4A-4D, non-isoform-selective inhibitors show narrow therapeutic windows, which might be widened by isoform-selective compounds [12]. Another direction for improvement of PDE inhibitor therapy is the development of inhibitors selective for other subtypes than PDE3, 4 or 5. PDE1 has shown promise as a therapeutic target, and the selective inhibitor lenrispodun (ITI-214) has recently entered phase II and III studies. In this review PDE1 is being discussed from molecular aspects to clinical entry for the use against cardiovascular disease.

1.1. Molecular aspects, enzyme kinetics and specificity issues of PDE1 and its inhibitors

The PDE1 family of dual cAMP / cGMP metabolizing enzymes consists of three isoform members PDE1A, PDE1B, and PDE1C, of which splice variants (1A1–12, 1B1–2, 1C1–5) have been detected [16,17]. PDE1 isoforms contain two calmodulin (CaM) binding domains in the N-terminal half of the enzyme, and a catalytic domain in the C-terminal part, responsible for metabolism of both cyclic nucleotides (Fig. 1) [5]. The 1A isoform variants differ in their N-terminal region, determining their activation by Ca2+ / CaM [17]. PDE1 is the only known Ca^{2+}-activated PDE isoform, and a classical approach to test their cAMP or cGMP metabolizing activity was to use cell lysates in the presence and absence of Ca^{2+} and CaM. PDE1 is active in the absence of Ca^{2+}, and upon binding of Ca^{2+} / CaM the maximal rate of metabolism of cyclic to non-cyclic nucleotide increases. The stimulation by Ca^{2+} / CaM is probably due to inactivation of an inhibitory sequence that is situated between the two CaM binding domains [6]. The increase in activity elicited by Ca^{2+} / CaM binding displays isoform-specific levels: eg. Vmax of cAMP degradation increases by 2.1 fold for PDE1A2 and by 2.7 fold for PDE1C4 [18]. Also the sensitivity to CaM and Ca^{2+} differs between the isoforms. For activation of PDE1A1 and A2, CaM showed an EC_{50}, or K_{CaM}, of resp. 0.3 nM and 4 nM [6,19]. Calcium sensitivity of CaM-PDE, measured in the presence of a fixed concentration of 244 nM

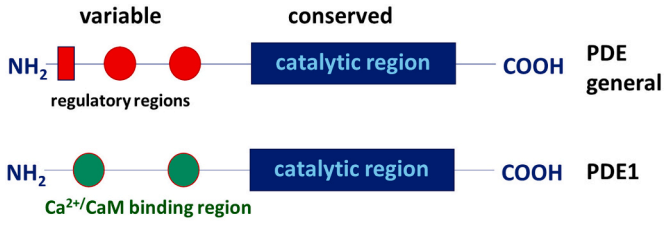

Fig. 1. PDE1 *vs.* general PDE structure. The catalytic C-terminal part is relatively conserved to accommodate conversion of either cGMP, cAMP, or both. The N-terminal part contains regulatory domains. In the case of PDE1 isoforms (1A1–2, 1B1, 1C1–4) these are Ca^{2+}/CaM binding domains, which upon binding inactive an inhibitory sequence. In other PDE subtypes these might be cGMP- (GAF), protein kinase A- or protein kinase B-regulated sequences.

CaM, is considerably higher. The EC_{50} of Ca^{2+} are 0.27 μM and 1.99 μM resp. for PDE1A1 and A2, and PDE1C1 has the lowest affinity with an EC_{50} of 3.01 μM [6,16]. *In vitro,* PDE1A displays a higher affinity for cGMP (K_m ~ 5 μM) than for cAMP (K_m ~ 112 μM), and the same is true for PDE1B (K_m ~ 3 μM and ~ 24 μM, respectively). PDE1C has a similar affinity for cAMP compared to cGMP (K_m ~ 1 μM) [20].

PDE1 gene expression patterns of the different isoforms vary from organ to organ, species to species, and between physiological and disease conditions [20]. Important for this review is the differential expression of the isoforms in the various cardiovascular tissues under healthy *vs.* disease conditions, which is responsible for the change in function of PDE1 and its role as a drug target. Therefore, the expression pattern will be discussed. Another important aspect is the specificity of the methods that were used to inspect the role of PDE1 and its isoforms. Unraveling the relative contribution of PDE1 *vs.* other PDE subtypes in vasomotor control was until recently problematic, and depended on the use of the alleged PDE1 inhibitor vinpocetin. Unfortunately, vinpocetin was show to be a poor tool with low selectivity, inhibiting other PDE subtypes, modulating calcium, potassium, and sodium currents, and blocking adenosine receptors [21]. Similarly, the specificity of 8-methoxymethyl 3-isobutyl-1-methylxanthine was found to be questionable [21]. This should be taken into consideration when reading the remainder of this review. The degree of selectivity found for a certain inhibitor appears to depend on whether cell lysates or whole cell or organ preparations are being used for specificity test [21]. In the latter preparations, the selectivity was found to be lower than in early studies that used cell lysates. More recently PDE1-specific inhibitors and genetic strategies to suppress specific PDE1 isoforms have been developed.

2. PDE1 in vascular tissue

2.1. Vascular expression pattern

Vascular expression of *PDE1* differs between species, vascular bed and (patho)physiological conditions. In the rat, PDE1A, PDE1B and PDE1C mRNA was found in aorta, whereas mesenteric arteries only showed PDE1A and PDE1B mRNA. Rat skeletal muscle arterioles and venules express all three subtypes according to similar patterns [22]. Some differences in expression levels were found between juvenile and adult rats and between sexes, but these differences are not large enough to *a priori* expect any difference in the functional role of PDE1 between these groups [22]. In mice, *Pde1a* and *Pde1c* transcripts were detected in aorta, and PDE1A protein was demonstrated [23,24]. Mouse aortic Pde1c is strongly upregulated by conditions that evoke neointima formation, and by aging; both conditions that are characterized by hypertrophy of the vascular wall and by a shift from the quiescent, contractile to the synthetic, proliferative phenotype of VSMCs [23,25].

With respect to larger mammals, PDE1 has been detected in the media of porcine pulmonary artery [26]. In humans, PDE1A and B, but not C, were detected in cerebral artery [27]. All isoforms were detected in human aortic VSMC, PDE1A and B in quiescent VSMC, as characterized in fresh aorta homogenates, and PDE1A and C in cultured, proliferating aortic VSMC [28]. PDE1C also is seen in VSMC of atherosclerotic plaque [28]. Additional stimulation of proliferation by culturing human aortic VSMC on monomeric collagen further increases PDE1C, whereas PDE1A totally disappears [29]. Fetal aorta expresses PDE1C, and this is replaced shortly after birth to PDE1A and B [29].

Also endothelial cells (EC) express PDE1. In cultured rat skeletal muscle microvascular EC PDE1A is expressed, with a slightly higher expression in males than in females [30]. Early studies in porcine and bovine aortic EC show absence in early primary cultures, when the cells show the typical cobblestone appearance. However, in bovine arterial EC from a high passage PDE1 activity emerges [31–33]. In addition, PDE1 emerges in cultured bovine aortic EC when the cells pass from a cobblestone to a spindle shaped phenotype, which is interpreted as a transition toward an angiogenic phenotype [34].

In summary, the expression of PDE1 isoforms appear to shift upon induction of processes that lead to a redifferentiation to the fetal, proliferative or angiogenic phenotype (Fig. 2). In VSMC PDE1C emerges under proliferative conditions, which in all likelihood is important for the pathophysiology of vasomotor dysfunction and aberrant remodeling. In EC, PDE1 appears under proliferative conditions as well, and the involvement of the different isoforms still needs to be characterized. For EC the functional role of PDE1 is unknown, and will not be further discussed.

2.2. PDE1 in VSMC, effect on vasomotor function and blood pressure

In the quiescent, contractile phenotype PDE1A is the predominant isoform, and it is localized in the cytoplasm. Therefore, PDE1A was presumed to be the primary isoform involved in vascular tone regulation, which takes place through modulation of actin-myosin interaction [28,35]. Both cAMP and cGMP might be involved in the vasomotor effects of PDE1. With respect to cGMP regulation, PDE1 shares its task predominantly with PDE5, as was shown in rabbit aorta in an early study [36]. The production of cGMP in vascular tissue can either be prompted by binding of nitric oxide (NO) to soluble guanylyl cyclase, or of natriuretic peptides to their membrane receptors, which are particulate guanylyl cyclases [3]. In relation to cAMP regulation, PDE1 might share tasks with PDE2, PDE3 and PDE4, but until now there is hardly any evidence that PDE1 modulates vasomotor responses evoked through cAMP. Very early studies have shown though that cAMP metabolism is indeed partly taking place through PDE1 activity, although at the time this the relevance for vasomotor function was not tested [28,34]. Unraveling the relative contribution of PDE1 *vs.* other PDE subtypes in vasomotor control was until recently problematic, and depended on the use of the alleged PDE1 inhibitor vinpocetin. For example, vinpocetin was shown to increase acetylcholine-induced NO-mediated cGMP production in vascular tissue [37], and effect that is mediated by the muscarinic M3 cholinergic receptor. Also, it increased vasodilation in mouse aorta [38]. Vinpocetin was also shown to decrease vasoconstrictions, and this effect was prompted by exposure of vascular tissue to Angiotensin II (Ang II). In agreement, Ang II increased PDE1A1 expression and PDE1-mediated cGMP metabolism in rat cultured VSMC [39]. Similarly, Ang II infusion in rats, which leads to hypertension, induced expression of PDE1 in aorta and mesenteric artery. This was accompanied by an appearance of an inhibiting effect of vinpocetin on phenylephrine-induced vasoconstriction, as measured *ex vivo* in organ baths [40]. In contrast, vasoconstrictions to $CaCl_2$ were similarly blocked by vinpocetin in angiotensin II- *vs.* vehicle-infused rats. Apparently, the vasoconstriction-inhibiting effect of PDE1 is an interplay between its expression levels and intracellular Ca^{2+} levels, perhaps involving compartmentation of Ca^{2+} entry, as this differs between angiotensin II and $CaCl_2$ [40]. Conversely, also infusion of the vasodilator nitroglycerin in rats induced PDE1 increase when infused for 3 days, a time period long enough to induce insensitivity to nitroglycerin [39]. Vinpocetin partly reversed this insensitivity. Therefore, PDE1 was proposed to play a role in nitrate tolerance, a well-known clinical problem during the treatment of recurrent angina pectoris [39].

Interestingly, in pulmonary hypertension induced by prenatal hypoxia in mice, the expression of muscarinic M1 receptor is induced in VSMC of pulmonary artery [37]. This receptor increases cGMP metabolism and decreases NO-mediated vasodilation. Both effects can both be blocked by 10^{-5} M vinpocetin. Therefore, an interaction between M1 and PDE1 was proposed. Considering the use of vinpocetin in this study, this interesting paradigm remains hypothetical. Yet, studies in pulmonary arterial VSMC from humans and from hypoxia-treated mice or monocrotaline-injected rats showed a strong increase of PDE1 in pulmonary hypertension; PDE1C in the human tissue and PDE1A in the rodent models [41]. A chronic treatment with the PDE1-inhibiting compound 8-methoxymethyl 3-isobutyl-1-methylxanthine lowered pulmonary arterial pressure and attenuated pulmonary arterial and right ventricular remodeling. he effects of PDE1 inhibition in pulmonary hypertension remain to be confirmed with truly specific compounds. More reliable insight in the functional role of PDE1 was obtained after the recent development of selective inhibitors, and with the help of genetic models. Selective inhibitors have usually been designed to target the C terminal catalytic part, which is the most suitable binding site for this purpose as it allows targeting of conserved functional elements whilst creating selectivity with the use of non-conserved amino acids on the basis of computational design [42] (Table 1: overview of the

Table 1
Properties of characterized, selective PDE1 inhibitors mentioned in this review.

Name	IC_{50} 1A:1B:1C (nM)	references
BTTQ	1: 0.45: 0.14	[45]
IC86340	440: 210: 60	[42]
Lenrispodun	0.034: 0.380: 0.037	[8,84]
Lu AF41228	170: 39: 78	[44]
Lu AF58027	13: 45: 1.4	[44]
PF-04822163	43: 9: 38	[85,86]

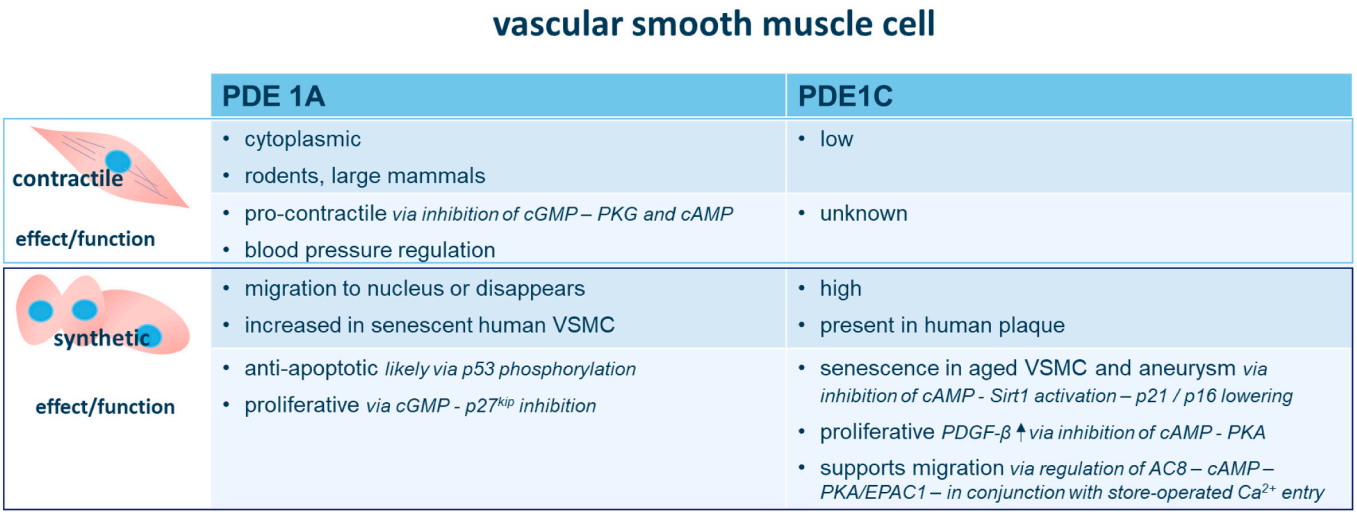

Fig. 2. Role of PDE1A and PDE1C isoform in vascular smooth muscle cells. Expression pattern (darker shaded rows) and functional consequences (lighter shaded rows) are described. The connected signaling cascade is written in italics.

inhibitors mentioned in this review). Genetic models comprise the use of commercially available si/shRNA, and *Pde1a* and *Pde1c* knockout mouse models generated by laboratories at the University of Rochester and the Mayo Clinic Rochester, USA [7,43].

2.3. Pharmacological studies on vasomotor function with subtype-specific PDE1 inhibitors

The PDE1-specific inhibitors Lu AF41228 and Lu AF58027 relaxed isolated rat mesenteric arteries dose-dependently in an NO- and cAMP-dependent manner, confirming the role of PDE1 in vasorelaxation *ex vivo* [44]. *In vivo*, infusion of these inhibitors reduced blood pressure, and increased heart rate [44]. Similarly, the PDE1-selective inhibitor BTTQ dose-dependently relaxed isolated rat aorta, and lowered blood pressure in normotensive Sprague-Dawley, Spontaneously Hypertensive and Dahl Salt-Sensitive rats [45]. All *in vivo* studies in rats had a short-term, bolus injection design. Similar to rats, in dogs short-term infusion of the PDE1-specific inhibitor lenrispodun (ITI-214) decreased peripheral vascular resistance [46]. In mice, a 7-day infusion protocol with PDE1 inhibitor IC86340 caused a small, biphasic decrease of blood pressure with a maximal effect at day 5 [47].

In genetically modified mouse models we found a relationship between PDE1 and aging. *Ercc1*$^{\Delta/-}$ mice undergo strongly accelerated aging, amongst which in vascular tissue. In *Ercc1*$^{\Delta/-}$ mice aging is strongly accelerated due to an increased DNA damage response that has been induced by introduction of a null allele (−) and a deletion mutation in exon 7 (Δ) in of the *Ercc1* gene, which causes an increase of transcription stalling DNA lesions due to a defective ERCC1 endonuclease [38]. Amongst other vascular aging features, NO-mediated vasodilator function is strongly decreased in *Ercc1*$^{\Delta/-}$ mice compared to wild-type (WT) mice, already at the age of 12 weeks. Both PDE1A and C mRNA was detected in aortic tissue of *Ercc1*$^{\Delta/-}$ and wild-type littermates [23]. *Ercc1*$^{\Delta/-}$ mice show increased PDE1C protein in lung tissue compared to wild-type littermate mice, whereas PDE1A did not differ [23]. Vinpocetin potentiated *ex vivo* measured aortic vasodilations to the NO donor sodium nitroprusside remarkably stronger in *Ercc1*$^{\Delta/-}$ mice than in WT littermates [23]. Recently, we showed that the specific PDE1 inhibitor ITI-214 (lenrispodun) significantly improved NO responses in *Ercc1*$^{\Delta/-}$ mouse, and not in WT mouse, aortic rings measured *ex vivo* in organ baths [48], thus confirming that PDE1 effects on vasomotor function are increased in aging. In addition, we corroborated this result in smooth muscle-specific *Ercc1* knockout mice [49]. Regarding blood pressure, lenrispodun, given for 8 weeks, only lowers blood pressure in *Ercc1*$^{\Delta/-}$ and not in WT C57b/6::FVB mice, which confirms the physiological importance of the increase of PDE1 participation in aged mice in an *in vivo* setting [48].

2.4. PDE1 dominates over PDE5 in aging-induced vasomotor dysfunction

Interestingly, PDE5 inhibitor sildenafil did not have an effect on NO-mediated vasodilation in smooth muscle-specific accelerated aging mice [49], suggesting that in aged mouse VSMC PDE1 dominates over PDE5 when it concerns the modulation of NO-mediated responses. This domination can have diverse explanations next to the already mentioned increase of PDE1 expression. Firstly, PDE5 requires binding of cGMP to its GAF domain to be activated, and in aging vascular NO-stimulated cGMP production is low [38,50]. Secondly, PDE5 activation appears to require co-localization with dimerized PKG [51], at least in cardiomyocytes. Several publications combined suggest that PKG dimerization requires a prolonged cGMP increase and subsequent oxidation of PKG by H_2O_2 [52–54]. The low cGMP levels in aged blood vessel would not permit PKG-mediated PDE5 activation. Thirdly, the relative abundance of vasoconstrictor over vasodilator stimuli in aged blood vessels may favour increased Ca^{2+} levels in aging, activating PDE1. Aging also leads to disturbed Ca^{2+} regulation in VSMC [55]. Overall, these studies imply that there a clearly distinctive role of PDE1 *vs.* PDE5 in vasomotor control. The potential involvement of the nanodomains herein will need further interrogation.

2.5. Vasomotor function in genetic models of PDE1 inhibition

With regard to genetic models, suppression of cytosolic PDE1A in quiescent human VSMC reduced myosin light chain kinase phosphorylation, which is in line with the hypothesis that PDE1A plays a role in myosin-actin function [35]. Accordingly, *Pde1a* null mice displayed low blood pressure [24]. This might not *per se* reflect a vascular role, but might implicate renal effects: *Pde1a* KO mice showed an aberrant renal phenotype that is reminiscent of cystic kidney disease. If, and how, the effects of *Pde1a* knockout in the kidney translate in blood pressure decrease has not been explored. *Pde1a* or *Pde1c*, but not *Pde1b*, deletion also aggravated renal cystic disease on a *Pkd2*$^{WS25/-}$ background, a model for this disease [24,43]. Genetic removal of *Pde1c* in mice on a normal WT background has no effect on blood pressure, which is in agreement with the absence of PDE1C in the quiescent VSMCs and the absence of a role of PDE1 in non-aged mice [7,56,57]. The effect of *Pde1a* and *1c* on an aged background has not been investigated.

In summary, PDE1 is involved in modulation of constriction and of relaxation of VSMC in various species. This is associated with blood pressure effects. This role of PDE1 involves cGMP-mediated effects, although scarce evidence implicates cAMP as well. The PDE1 isoform involved depends on vessel type, condition and animal species. PDE1A can be increased and thus involved in vasomotor modulation by angiotensin II or prolonged nitroglycerin exposure. PDE1A appears to be involved in rodent models of pulmonary hypertension. In contrast, in human pulmonary hypertension PDE1C is increased. In addition PDE1C appears to be activated by aging in the mouse vasculature. Long term treatments with selective PDE1 inhibitors in might be interesting for treatment of hypertension or perfusion problems, especially in elderly. PDE1 inhibitor lenrispodun is in clinical development in patients with neurodegenerative disease and heart failure (www.intracellulartherapies.com) [58]. Interestingly, a single oral dosage of lenrispodun caused a modest lowering of blood pressure in middle-aged heart failure patients [58]. No long-term blood pressure studies have been performed in other animals than in *Ercc1*$^{\Delta/-}$ and littermate mice. Hence, lenrispodun is a clinically applicable drug that might be suitable for studies in hypertensive patients.

3. PDE1 in VSMC, role in vascular structural remodeling

PDE1A migrates to the nucleus when inducing the synthetic phenotype, PDE1B disappears, and PDE1C emerges (Fig. 2). Suppression of PDE1C with antisense oligonucleotides inhibits proliferation of cultured human VSMC [29]. Deletion of the *Pde1c* gene drives VSMC back into the contractile state, as was observed in cultured mouse cells [25]. These changes implicate PDE1 isoforms in structural remodeling of the vasculature. In this context their role has been explored in arteriosclerosis and aneurysm formation.

3.1. Pharmacological studies on the role of PDE1 in vascular remodeling

Several lines of evidence from pharmacological studies suggested the role of PDE1 in vascular remodeling. Vinpocetin partly prevented neointima formation in a diabetic rat balloon injury model [59]. Reduced production of reactive oxygen species, of proliferation and of resistance to apoptosis was observed in vinpocetin-treated cultured VSMC [59]. In the ApoE$^{-/-}$ mouse model of atherosclerosis, a 12-week treatment with vinpocetin reduced plaque lesion area and necrotic core, and increased fibrous cap thickness, all without affecting low and high density cholesterol or triglyceride levels [60]. In both arterio- and atherosclerosis the attenuation of Akt/NFκB signaling was implicated, and in the atherosclerotic model this might relate to effects on monocytes adhesion. However, although PDE1B was prominently present in blood

mononuclear cells, the attenuation of PDE1B expression in cultured macrophages did not affect Akt/NFκB [60]. Intriguingly, in another study it was shown that vinpocetin exerted the effect on Akt/NFκB independently from PDE, through a mechanism that might involve oxidized LDL cholesterol receptor 1 [61]. Therefore, vinpocetin appears to employ multiple mechanisms when reducing arterio- and atherosclerosis, and it is unclear if this involves PDE1 inhibition.

3.2. Vascular remodeling and implicated signaling pathways in genetic models of PDE1 inhibition

More specific evidence was obtained from knockout models. *Pde1c−/−* mice show less neointima formation after wire injury in carotid artery than WT mice [25]. *Pde1c−/−* deletion also decreased proliferation and migration of mouse cultured proliferative VSMC. Key signaling pathways modulated by PDE1 isoforms in the described growth and migration effects were identified. PDE1C, but not PDE1A, increase, as induced by transfer of mouse VSMC to conditions that promote the transition from the quiescent to the proliferative phenotype, promotes proliferation of VSMC by stabilizing the platelet-derived growth factor receptor (PDGF) β through inhibition of receptor endocytosis [25]. A local interaction between PDE1, PDGF-β and LDL receptor-related protein 1, which is involved in endocytosis of multiple receptors, was found to be responsible [25]. This process was shown to be mediated by cAMP/PKA and to be independent of Epac I, and involvement of cGMP/PKG was shown to be unlikely. Conversely, PDE1A migrates from the cytoplasm to the nucleus when transferring VSMC to proliferation-promoting conditions. *In vivo* this is observed as a nuclear localization of PDE1A in neointimal and cytoplasmic orientation in medial VSMC in the rat carotid balloon injury model, the rat low-flow neointima formation model and in hypertrophied human coronary artery [35]. PDE1 inhibition with the selective blocker IC86340 or suppression of PDE1A with small hairpin RNA increased apoptosis of cultured rat aortic VSMC but reduced proliferation [35]. This was associated with an increase of cell cycle suppressor $p27^{kip}$, a decrease of the cell cycle stimulating protein cyclin D, and phosphorylation of p53, which can result into apoptosis. The activation of the p27 – cyclin D signaling was reproduced by cGMP and not by cAMP [35]. Thus, PDE1A and PDE1C both play a role in vascular hypertrophy, resp. through cGMP and cAMP. The role of the proposed mechanisms in atherosclerosis have not yet been clarified, and also there are no studies describing the effect of chronic treatment with PDE1-selective inhibitors on atherosclerosis.

3.3. Role of PDE1 in VSMC migration: specific cAMP and Ca^{2+} interactions

Apart from proliferation and inflammation, migration of VSMC is another important event in vascular hypertrophy. VSMC of the synthetic phenotype, as prompted eg. by endothelial denudation, migrate from the arterial tunica media toward the lumen to form part of the neointima. To migrate, VSMC polarize, forming leading edge protrusions (LEP) at the side pointing toward the migration direction [62]. In cultured human arterial VSMC of the synthetic phenotype it was shown that cAMP-mediated LEP formation takes place *via* EPAC1 [62]. This LEP formation was inhibited by the PDE1 inhibitors PF-04827736 and a compound designated “C33” (not to be mistaken by the commercially available PDE9 inhibitor), and by PDE1C siRNA. In a subsequent study, it was shown that in PDE1C plays a role in regulation of store-operated Ca^{2+} entry by the Ca^{2+-}activated AC8 [63]. Store gated Ca2+ entry activates AC8 to increase produce cAMP, and subsequently this inhibits the store gated Ca2+ entry through inactivation of Ca2 + −selective, Ca2+ release-activated Orai channels [63]. PDE1C, AC8 and Orai1 were found to be localized in LEP, and a direct interaction between PDE1C and Orai1 was demonstrated. In addition, PKA was found to be another binding partner, involving A-kinase anchoring protein 79 as a scaffolding protein [63]. Summarizing the interactions and its functional role, it was proposed that PDE1C likely acts within an AKAP-based, PKA-containing signalosome to regulate HASMC LEP formation and that ADCY8 was dominant in providing the source of cAMP [63].

4. Role of PDE1 in vascular matrix remodeling and aneurysms

Apart from having a role in proliferation and migration, PDE1 also affects extracellular matrix deposition. In proliferative rat aortic VSMC, PDE1 inhibition with the selective inhibitor IC86340 stimulates lysosome-mediated degradation of collagen I, decreasing its basal intra- and extracellular levels [64]. This observation was reproduced in cultured saphenous vein [64]. This effect on lysosomes involved cyclic nucleotide-gated ion channels rather than PKA, PKG, their target vasodilator-stimulated phosphoprotein, or Epac. The channels were activated by cAMP that was produced by soluble adenylate cyclase [64].

Changes in matrix deposition by VSMC can have various consequences in the vasculature. It can modulate vascular stiffness. In $Ercc1^{\Delta/-}$ progeroid mice the increase of vascular stiffness was not changed by chronic lenrispodun treatment [48]. However, there was no obvious histological evidence for a change in extracellular matrix in this model, and therefore the increased vascular stiffness might involve other causes. The role of PDE1 in vascular stiffness requires closer inspection, and probably demands a comparison of models in which increased vascular stiffness is caused by different mechanisms.

Another consequence of matrix modulation can be weakening of wall structure, such as in the case of aneurysm formation. Several forms of evidence connect cAMP and cGMP to aneurysm formation. In mice, aneurysm formation was shown to be promoted by genetic deletion of Gsα G-protein, which drives cAMP signaling, and in humans the occurrence of the PRKG1 c.530G > A mutation, which leads to gain-of-function of PKG-1, predisposes to aneurysm formation [65,66]. In parallel there is evidence for a role of PDE1. In the chronic angiotensin II infusion and elastase-induced aortic aneurysm model in $ApoE^{-/-}$ mice PDE1C expression was increased. Pde1c deletion or PDE1 inhibition with IC86340 attenuated aneurysm formation [67]. The outcome is in paradox with the observation that PDE1 inhibition inhibited VSMC proliferation and collagen formation in other studies, as described above, because this should lead to further weakening of the arterial wall. The contradiction suggests that the effect of PDE1 inhibition differs according to the encountered circumstances. Aging might be an important factor in this respect.

5. A potential role of PDE1 in senescence

Cellular senescence is a state of cell cycle arrest, involving inhibition of cyclin-dependent kinase, predominantly by the endogenous inhibitors p16 and p21, is accompanied by functional loss, and occurs mainly as a response to DNA damage response [68]. Senescent cells show an increased release of inflammatory factors, which negatively affect neighbouring cells, and in endothelial cells senescence is associated with a loss of NO release [69]. In recent years, it has been shown that removal of senescent cells can extend life span and improve aging variables [70]. So-called senolytic compounds have been developed that remove senescent cells. There is some evidence that treatment with such compounds can improve endothelium-dependent and -independent vasodilations [71]. Furthermore, overexpression of the deacetylase sirtuin-1 (SIRT-1), an important regulator of p21, attenuates aneurysm formation in the angiotensin II infusion / $ApoE^{-/-}$ model [72]. Therefore, reduction of senescence might improve vascular aging.

Our work in $Ercc1^{\Delta/-}$ progeroid mice suggests that aging, induced by DNA damage, increases PDE1C [23]. In addition, we showed that PDE1C increased in cultured human VSMC that were exhaustively passaged, and that thus developed senescence, as demonstrated with the markers p16, p21 and senescence-associated β-galactosidase [23]. We showed that vinpocetin treatment decreased p16 and p21 expression in these

cells, suggesting a senolytic-like effect [23]. Whether this plays a role in the vascular protective effect that was shown with chronic lenrispodun in *Ercc1*$^{\Delta/-}$ mice awaits confirmation [48]. Interestingly, PDE5 inhibition again showed a differential effect as it only decreased p21 [23]. Genetic removal or adenoviral vector-induced overexpression of PDE1C respectively decreased and increased senescence in cultured mouse aortic VSMC [67]. In abdominal aorta aneurysm models, knockout of PDE1C reduced the DNA damage marker γ-H2AX, which can be, but not necessarily is, associated with senescence [67]. The anti-senescent effect of PDE1C deletion in cultured VSMC was SIRT-1-dependent. Sirt-1 activation by PDE1C inhibition did not involve PKG, PKA or Epac. Rather, it might involve binding of cAMP directly to SIRT-1, as the nucleotide and the enzyme were shown to co-localize in close proximity [67].

5.1. Relevance in human vascular aging

Evidence of the involvement of PDE1 isoforms in human vascular aging comes from genetic epidemiological studies. In cardiovascular data from the International Consortium of Blood Pressure Genome-Wide Association Studies and the AortaGen Consortium we found that various single nucleotide polymorphisms in the *PDE1A* gene associated with diastolic blood pressure and common carotid intima-media thickness in a candidate gene approach [23]. Similarly, in a hypothesis-free approach *PDE1A* single nucleotide polymorphisms associated with diastolic blood pressure and mean arterial pressure [73]. *PDE1C* polymorphisms did not associate with vascular aging variables in these studies. This may simply reflect the absence of functional mutations in the gene, and does not dismiss PDE1C as a relevant protein.

6. PDE1 in ventricular function and remodeling

6.1. Role of cyclic nucleotides in the cardiac ventricle

The role of cyclic nucleotides in cardiac modulation has been intensively studied, and is excellently summarized elsewhere [74]. To sketch the context, a small overview is provided here. Activation of PKA by cAMP in myocytes leads to modulation of contractility and relaxation through regulation of Ca^{2+} entry, cycling and responsiveness by phosphorylation of various targets, including voltage-gated L-type calcium channel, phospholamban, ryanodine receptor and proteins of the contractile apparatus [74]. The modulation of Ca^{2+} can also involve Epac, which acts through calcium-calmodulin dependent kinase-II, calcineurin, and GTP-binding proteins [74]. Furthermore, cAMP facilitates excitation - contraction coupling and sarcomere function. [46] Also, heart rate modulation can occur through hyperpolarization-activated, cyclic nucleotide gated-channels. *Via* CREB cAMP can modulate the myocardium through transcriptional effects, which leads to chronic remodeling [74]. Being such a versatile messenger, cAMP modulation may be both beneficial or detrimental, depending on the conditions in the myocardium and the duration of the cAMP modulation. As an example, acutely, activation of β-adrenergic receptor (β-AR) - cAMP signaling is procontractile. However, β-AR activation also induces apoptosis, and chronic stimulation even leads to hypertrophy. Adenosine-derived cAMP signaling can on the other hand attenuate many of these detrimental effects [57].The observation that different cAMP-increasing stimuli can exert contrary effects implicates compartmentation. A thorough knowledge of the role of the various cAMP-metabolizing PDE subtypes and isoforms in nanodomains of the heart is therefore invaluable.

Through PKG cGMP can modulate transient receptor potential channels, G proteins, and the contractile apparatus in cardiac myocytes and thus modulate contraction and stiffness of the myocardium [75,76]. An increase in cGMP content in response to activators of membrane-bound guanylyl cyclases are associated with positive chronotropic and inotropic effects, whereas an increase in cGMP content in response to activators of soluble guanylyl cyclases are associated with negative inotropic effects [77]. Increasing cGMP by PDE inhibition can be used to prevent cardiac hypertrophy in animal models, as demonstrated for PDE5 in mice [78]. In cardiac fibroblasts, the increase of cGMP – PKG induced by atrial natriuretic peptide inhibits transformation to myofibroblasts, collagen formation, and proliferation [79]. This involves inhibition of mothers against decapentaplegic homolog (Smad) 3 – transforming growth factor (TGF) β signaling.

To control the versatile, if not paradoxical, effects of the cyclic nucleotides the heart contains several PDE subtypes, of which PDE1 is the only Ca^{2+} – activated form [20]. Since Ca^{2+} increase is responsible for contraction, PDE1 is an obvious subtype to study in relation to myocardial contractile function. However, PDE1 has also been implicated in hypertrophy, apoptosis and fibrosis, as outlined in the next sections.

6.2. Cardiac expression and distribution of PDE1

The PDE1 isoforms are differentially expressed in the myocardium, and also differ between species. PDE1A is expressed in human, bovine, canine and rodent heart, and is increased in cardiac disorders, notably in human heart failure, and in rodent heart failure models of transaortic coarctation (TAC), during chronic infusion of isoproterenol or of angiotensin II, and after myocardial infarction [20,47,57,80]. PDE1B is hardly detectable in all species. In the myocardium PDE1C is present and its protein levels appear to dominate over those of PDE1A in humans, dogs and rabbits, whereas being relatively low in rats and mice [46,81]. In human cardiac myocytes PDE1C is localized along the M- and *Z*-lines, creating the typical striated pattern [81], and it can also be found in the myocyte nuclei. This is suggestive for a role in both contractility as well as remodeling.

The levels of PDE1 isoform mRNA, or changes thereof, eg. after induction of heart failure, do not consistently correspond with changes in protein levels. For example, abundant *Pde1c* mRNA was detected in mouse heart, but PDE1 protein appears to be virtually absent under baseline conditions [47]. This discrepancy can also be found in human and dog myocardium [46]. For example, in human ventricle of failing hearts PDE1C mRNA was found to be increased compared to healthy myocardium, but protein levels were unchanged [46,57]. As a result, results obtained with PCR and western blot are not always interchangeable. In contrast, expression of *Pde1c* in mice is increased by pressure overload with TAC, and both visible at the level of mRNA and protein (Fig. 3) [57]. Presumably this change in *PDE1C* takes place in cardiac myocytes, because cultured cardiac fibroblasts did not show *Pde1c* mRNA, also not after profibrotic stimulation with TGF-β [57].

The heart mainly contains VSMC, EC, cardiac myocytes and fibroblasts. The role of PDE1 in coronary vascular cells has not been studied, with the exception that the upregulation of PDE1A and C and the senescence-inhibiting effect of vinpocetin were demonstrated in cultured human coronary vascular smooth muscle cells [23]. Until now studies have focussed on cardiac myocytes and fibroblasts [20]. In cardiac myocytes PDE1 was shown to contribute to remodeling and contractility, and in fibroblasts it controls extracellular matrix production.

7. PDE1 in cardiac myocytes

7.1. Hypertrophy

Cultured neonatal and adult rat ventricular myocytes IC86340 attenuated phenylephrine-induced hypertrophy and mRNA and protein production of atrial and brain natriuretic peptide (ANP, BNP), two well-known cardiac hypertrophy markers (Fig. 3) [47]. Genetic PDE1A, but not PDE1C, downregulation had similar effects and abolished the IC86340 effect. Also Ang II induced a hypertrophic response in rat cardiac myocytes that was modulated by PDE1A [47]. The role of

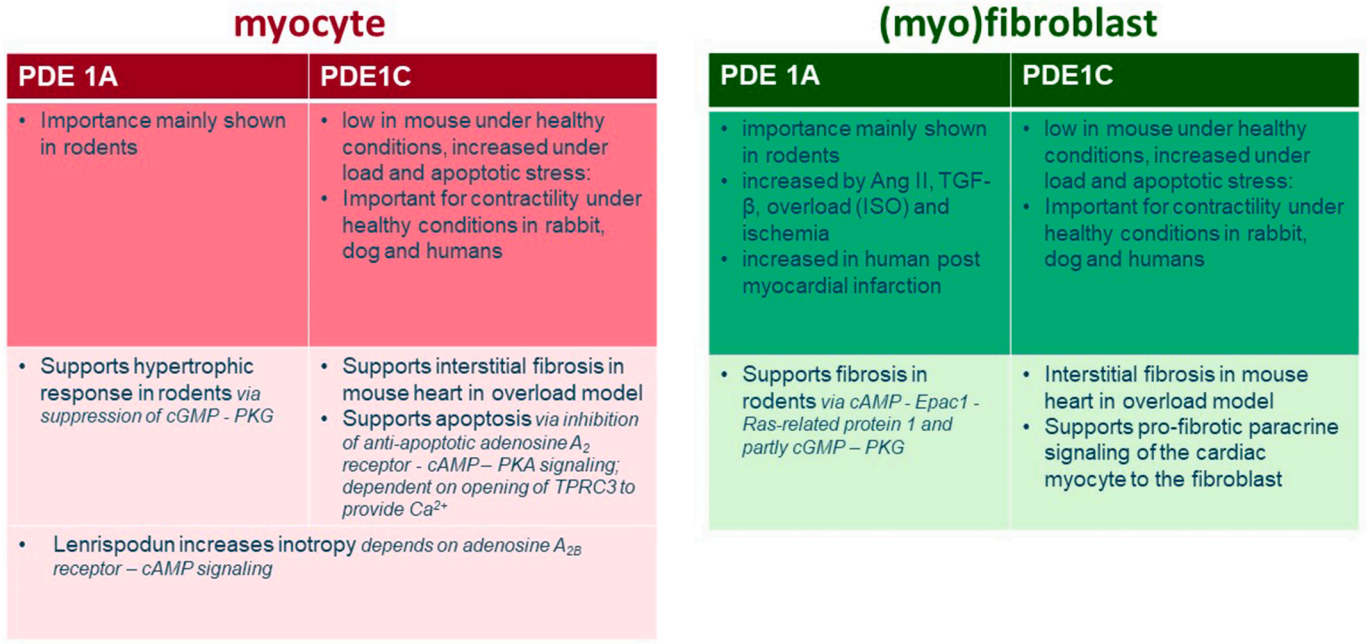

Fig. 3. Role of PDE1A and PDE1C isoform in cardiac myocytes (red shades) and (myo)fibroblasts (green shades). Expression pattern (darker shaded rows) and functional consequences (lighter shaded rows) are described. The connected signaling cascade is written in italics. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PDE1A in myocyte hypertrophy depended on cGMP – PKG signaling, and not cAMP – PKA.

In mice, TAC induced a PDE1C increase in cardiac myocytes, which was accompanied by a dilated hypertrophy, fibrosis, apoptosis and a functional remodeling marked by reduced fractional shortening [57]. *Pde1c* knockout decreased these detrimental effects of TAC. The anti-fibrotic effect was limited to interstitial fibrosis, and did not involve perivascular fibrosis. It was also demonstrated that the effect was likely to be mediated through paracrine modulation of fibroblasts by cardiac myocytes.

7.2. Apoptosis

In cultured mouse cardiac myocytes *Pde1c* knockout abolished Ang II- and isoproterenol-induced apoptosis, and this was cAMP – PKA, but not cGMP, −dependent, and involved PI3K/AKT (Fig. 3) [57]. *Pde1c* knockout also attenuated doxorubicin and H_2O_2-induced cardiac myocyte apoptosis [7]. In contrast, *Pde1a* knockout did not affect any of the apoptotic stimuli [7]. The effect of PDE1C removal was dependent on adenosine A_2 receptors. IC86340 or *Pde1c* knockout attenuated Ang II-induced apoptosis, and this anti-apoptotic was abolished in the presence of A_2 receptor antagonist ZM241385 or after incubation with A_2 receptor antisense oligonucleotides [7]. PDE1C inhibition and A_2 receptor stimulation acted in synergy. Adenosine increases cAMP through the A_2 receptor. The A_2-dependency was not mimicked by a different cAMP-dependent cardiac protective stimulus, the activation of prostaglandin receptor EP4, suggesting signal selectivity for PDE1C modulation of cardiac myocyte apoptosis [7]. Further exploration revealed the potential mechanism for this selective interaction: Ang II opens transient potential receptor channel 3 (TPRC3), and the resulting increase of Ca^{2+} activates PDE1C to lower cAMP, thus suppressing an anti-apoptotic effect sustained by A2 receptor activation. This mechanism involves a close spatial interaction between the implicated proteins, and was shown to be active in a mouse model of doxorubicin-induced cardiac toxicity.

7.3. Contractility and hemodynamic control

With respect to the role of PDE1 in myocardial contractility, it was shown that in the healthy and failing myocardium of respectively naïve and 3-week-tachypaced dogs the PDE1 inhibitor lenrispodun increased inotropy [46]. Chronotropic and lusitropic effects were also observed. Lenrispodun and the β-adrenergic agonist dobutamine, of which the latter elevates cardiac contractility through increase of Gs protein-mediated cAMP production, acted additively in dogs, not synergistically, suggesting that their effects took place independently. In agreement, lenrispodun increased contractility in rabbits, and this effect was not abolished by blockade of β-adrenergic receptors [46]. In contrast, blockade of the adenosine A_{2B} receptor, which also promotes cardiac contraction through Gs – cAMP, fully reversed the inotropic effect of lenrispodun [46].

The inotropic effect of lenrispodun was not observed in mice. Taken together with the observation that compared to mouse myocardium the canine myocardium shows a PDE1 isoform expression pattern that is more similar to humans, *i.e.* predominance of PDE1C in dogs *vs.* PDE1A in mice, it is assumed that the mouse is not a model that is representative for humans. At least, this seems to be true in relation to myocardial contractility; when it concerns morphological remodeling, a PDE1C – adenosine interaction was also observed in mice (previous section).

8. PDE1 in cardiac fibroblasts

PDE1A protein is expressed in cultured rat cardiac fibroblasts, and is increased after 12 to 24 h of incubation with Ang II. This increase is accompanied by an increase of the relative contribution of PDE1 to total cGMP metabolism from 20% to 70% [80]. *Pde1b* and *c* mRNA remains unaffected. In these cells, IC86340 or shRNA-mediated decrease of PDE1A attenuated Ang II-induced pro-fibrotic markers type I collagen A, fibronectin, plasminogen activator inhibitor 1, and α-SMA [80]. Instead, suppression of PDE1C had no effect. Similar to rat fibroblasts, *Pde1c* deletion in cultured mouse cardiac fibroblasts did not affect the pro-fibrotic stimulus evoked by TGF-β [57]. Thus, cell culture experiments demonstrate a role of PDE1A in cardiac fibroblasts, and no role for

PDE1C. This is in sharp contrast with the earlier mentioned role of PDE1C in paracrine stimulation of fibrosis by cardiac myocytes. Thus, both isoforms play a role in fibrosis: PDE1A in fibroblasts themselves, and PDE1C *via* cardiac myocytes.

In vivo, isoproterenol infusion or left coronary artery ligation in mice increased the pro-fibrotic transformation of fibroblasts into myocardial fibroblasts [80]. Myofibroblasts displayed high levels of PDE1A, as in contrast to cardiac fibroblasts in healthy myocardium [80]. Similar results were obtained after Ang II infusion in rats and in human post-myocardial infarction tissue. Thus, the role of PDE1A in cardiac fibroblast under pro-fibrotic stimuli might be conserved across different animal species. IC86340 treatment decreased myocardial fibrosis in the mouse isoproterenol model.

Looking further into signaling cascades, cAMP participated in the anti-fibrotic effect of PDE1A inhibition through Epac1 - Ras-related protein 1 signaling and a preferential (*peri*)nuclear cAMP increase [80]. In addition, the anti-fibrotic effect was partly cGMP – PKG –dependent, which also showed an interesting association with a preferential increase of cGMP in the (peri)nuclear area [80]. The anti-fibrotic effect of cGMP did not involve TGF-β - Smad 2/3 signaling, a pathway known to be involved in fibrosis. In a study in rats, however, a reciprocal interaction between cAMP and TGF-β was found. When cultured adult rat cardiac myocytes were stimulated with TGF-β to become myofibroblasts, a decrease of cAMP levels occurred [82]. Conversely, an increase of cAMP by stimulation of adenylate cyclase with forskolin or administration of the PKA-specific agonist N6-Phe-cAMP attenuated TGF-β-induced expression of the myofibroblast marker α-smooth muscle actin, and of fibrosis markers [82]. The aforementioned evidence points at a close relationship between Ang II, TGF-β, PDE1A expression, and cAMP, and the contrasting result regarding cGMP – Smad2/3 is an invitation to further inspect this network of signaling molecules.

9. Clinical translation

The research demonstrating protective myocardial effects of PDE1 inhibition has led to the first reported clinical test related to cardiovascular application [58]. A safety study with escalating (10, 30, 90 mg) single oral lenrispodun dosages ($n = 9$ / dose) *vs.* placebo ($n = 8$) was conducted in patients with heart failure to study tolerability and the acute hemodynamic response. Adult male and female patients, aged 18 to 80 years, with chronic stable heart failure, EF $\leq$ 35%, New York Heart Association class II–III were enrolled, and were blinded for treatment.

Lenrispodun caused a modest but statistically significant blood pressure lowering with an average maximal drop in systolic pressure of 8 mmHg in the 90 mg group. Cardiac output increased by up to 35%, and mean left ventricular power index by up to 33% (90 mg dosage). Systemic vascular resistance and left ventricular afterload were reduced. The effects were mostly dose-dependent, and qualified as the result of an inodilator response [58].

Mild adverse events were reported, of which hypotension or orthostatic hypertension were the most relevant (1 patient in 10 mg, 2 in the 30 mg and 2 in the 90 mg group). There were no adverse effects on cardiac rhythm. No changes in plasma cAMP or cGMP levels were observed [58]. Thus, the lenrispodun effects in humans were in agreement with to those observed earlier in dogs and rabbits [46]. A further development of lenrispodun as a drug for the treatment of heart failure was envisioned [58].

10. "PDE1, 3, 4, 5: what's the difference?": compartmentation

The title of this section is a frequently heard remark, only to be nuanced by those who are more than just initiated. The concept of cyclic nucleotides regulation by PDEs cannot be appreciated without being informed about compartmentation. Without this knowledge cyclic nucleotide signaling and its role as a treatment target cannot be explored and exploited to its full extent.

The effect of compartmentation of PDE subtypes has recently been summarized for the cardiomyocyte [74]. Overall, PDE subtypes and isoforms can be found in different nanodomains, allowing the interaction with specific cyclic nucleotide production sites, e.i. receptor – G protein – adenylate/guanylate cyclase complexes, and in the case of PDE1 also with Ca^{2+} channels. As a consequence it can be expected that inhibitors of different PDE subtypes will have differential effects, even if it concerns elevating the levels of the same cyclic nucleotide. Here the consequences of compartmentation of PDE1 and related PDE subtypes are summarized, focussing on the studies that made direct comparisons between the roles of the subtypes. Some of those have been already described above, as will be indicated. This has been achieved in vascular smooth muscle cells, cardiac myocytes and cardiac fibroblasts with PDE1 *vs.* PDE3 or PDE5.

10.1. Compartmentation: different subcellular locations and functional partners

Until quite recently enzyme kinetic properties or PDE protein level were guiding in predicting the role of different PDE subtypes. For example, since the Km for cAMP degradation by PDE1 is around 1000× higher than for PDE3, it seemed logical to propose that in the low range of cAMP content PDE3 would dominate over PDE1, whereas at high this would not occur [18,81,83]. On the other hand, both cilostamide, or lenrispodun to did not noticeably change cAMP levels in cultured cardiac myocytes when measured in whole cell suspensions [46], despite the fact that both inhibitors have functional effect. Compartmentation of cyclic nucleotide increases that lead to high local concentrations can explain this phenomenon because it would mask increases measure in whole cell lysates. Often, however, cyclic nucleotide levels are measured in whole cell or tissue homogenates, if not on request after peer-review. In relation to specific functional effects of PDE inhibitors or agonists that act through cyclic nucleotides such measurements seem meaningless. Moreover, if PDE1 and PDE3 would be present in different compartments the Km is not a leading factor in determining the relative contribution of both PDE subtypes in functional modulation of cells.

The differential localization of PDE1 and PDE3 in *Z*- and M-lines and presence in different cell lysate fractions mentioned earlier in this review [81] hinted to compartmentation. Compartmentation might causes differences in functional partners, *i.e.* the signaling protein (receptor, enzyme, transporter) each specific PDE subtype is functionally associated with. This was indeed demonstrated. In an earlier mentioned study demonstrating the inotropic and lusitropic effect lenrispodun in rabbits and dogs [46], it was shown that PDE1 is coupled to adenosine A_2 receptor signaling, whereas PDE3 is coupled to β-adrenergic - cAMP signaling or forskolin-induced responses. The differential coupling of PDE1 and PDE3 is cell type-specific. In cardiac fibroblast, PDE1A enables the pro-fibrotic effect of β-adrenergic receptor stimulation, and also of Ang II. PDE3 inhibition did not have an effect on Ang II-induced fibrosis (β-adrenergic stimulation was not explored) [80].

Within the PDE1 subtype family, compartmentation and functional partnership is isoform-dependent. From studies in rat cardiac myocytes mentioned earlier, it has become clear that PDE1A is coupled to β-adrenergic receptors, thus regulating hypertrophy [47], and PDE1C to Ang II receptors, affecting apoptosis [7]. Also the finding in these studies that PDE1 inhibition decreases isoproterenol-induced apoptosis but not its inotropic effect is another example.

10.2. Compartmentation, but similar partners

The differential compartmentation of PDE subtypes does not necessarily entail the involvement of a different functional partner. In the case of rat cardiac myocytes, both PDE1 and PDE5 inhibition attenuate the hypertrophic effect of phenylephrine, and these effects were additive, not synergetic [47]. These findings suggested that within the same cell

PDE1A and PDE5A are likely coupled to unique cGMP pools despite the fact that in both cases attenuation of β-adrenergic receptor-mediated lowering of cGMP levels is involved [47].

A player in compartmentation of PDE1 *vs.* other PDE subtypes in nanodomains is Ca^{2+}. As mentioned above for migration of VSMC of the synthetic, PDE1 regulates cAMP formed by AC8 and thus store-operated Ca^{2+} entry in LEP. It was additionally shown that PDE3 and PDE4 are not involved in this interaction nor in PDE1C modulation of LEP formation, despite the fact that inhibition of PDE3 and 4 increase cAMP in VSMC [63]. Whether this is important in other functions of VSMC is not known. However, the earlier mentioned observation that the anti-vasoconstrictive effect of PDE1 inhibition on phenylephrine was dependent on Ang II pre-incubation, whereas the effect on CaCl2 was not [40], alludes to a differential role in various Ca^{2+} sources that are involved in vasoconstriction.

11. Summary and perspectives

PDE1 is increasingly presented as a target for treatment of cardiovascular disease. In vascular medicine PDE1 has a role in conditions during which hypertrophy is prompted, and therefore PDE1 inhibition might be beneficial in treatment of arteriosclerosis and atherosclerosis. Also, attenuation of vascular tone is a possible target. In mice, the tonus effect is associated with aging, in which the role of PDE1 increases. In rats this does not appear to be an issue. In humans, lenrispodun lowered blood pressure in heart failure patients. Implicitly, these individuals have and aged vasculature. Next to the vascular effect beneficial consequence have been shown for cardiac contractile function, hypertrophy, fibrosis and apoptosis. Therefore, the application of PDE1 inhibition to improve cardiovascular function, especially in elderly patients, is an interesting and important clinical possibility. Whether the effect in elderly extends to improved function of yet other organs, especially those related to human frailty at older age, such as brain, kidney, lung, and skeletal muscle, will be an important question to address.

A more fundamental question is the importance of nanodomains in the role of PDE1 isoforms, either compared with each other, or compared to other PDE subtypes. As explained, in these nanodomains PDE, AC, PKA and Ca^{2+} join to form a signaling unit. Herein, the adenosine A_2 receptor has emerged as a functional partner for PDE1, probably specifically PDE1C, in cardiac fibroblast and myocytes. A close spatial interaction involving Ang II-activated Ca2+ channels that locally increase Ca2+ levels and activate PDE1, which in turn attenuates local cAMP increase occurring after A_2 receptor stimulation, appears to be at play. In arterial VSMC AC8 has emerged as a functional partner of PDE1C in relation to store-operated Ca^{2+} entry regulation. Similarly, localized interaction with cGMP seems to occur, as witnessed by differential effects of PDE5 and PDE1 inhibition in cardiac myocytes and in young *vs.* aged blood vessels. The discovery of nanodomains has been an important breakthrough that will help us to understand cyclic nucleotide signaling to its full extent. What is still unclear is how the pathways run from these specific nanodomains all the way to the effector molecules without losing signal specificity. For example, PDE1-modulation of A_2 receptor signaling has been linked to both apoptosis and to contractility effects in cardiac myocytes. It is likely that these signals are separable, because a simultaneous inseparable occurrence of inotropy and apoptosis would not make sense. Therefore, the signals downstream of A2 receptor stimulation must be separated all the way to up to reaching the respective effectors. Similarly, in regulating smooth muscle contraction *via* cGMP, PDE1 and PDE5 appear to remain separated until the effector, the contractile apparatus, has been reached, otherwise maximal PDE1 and 5 inhibition would not act additively. Therefore, the nanodomains in which the PDE subtypes and isoform reside are merely the tip of the iceberg of signaling factors and effectors that lies beneath, or rather, downstream. An important start has been made, and the further unraveling will be a tremendous task that will deploy the coming decades.

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