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PDE1 Isozymes, Key Regulators of Pathological Vascular Remodeling

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Abstract

Pathological vascular remodeling is a hallmark of most vascular disorders such as atherosclerosis, postangioplasty restenosis, allograft vasculopathy, and pulmonary hypertension. Pathological vascular remodeling is a multi-cell dependent process leading to detrimental changes of vessel structure and eventual vessel occlusion. Cyclic nucleotide signaling regulates a variety of vascular functions ranging from cell contractility to cell growth. Cyclic nucleotide phosphodiesterases (PDEs), a large family of structurally and functionally distinct isozymes, regulate cyclic nucleotide levels and compartmentalization through catalyzing their degradation reaction. Increasing evidence has suggested that one of the important mechanisms for specific cyclic nucleotide regulation is exerted through selective activation or inhibition of distinct PDE isozymes. This review summarizes the work done to characterize the role and therapeutic potential of PDE1 isozymes in pathological vascular remodeling.

Introduction

Healthy vascular tissue is comprised of three layers, the tunica intima, media and adventitia. The intimal tissue is comprised of a layer of endothelial cells (EC) around the lumen of the vessel acting as a barrier to particles in the blood and mediator of vessel contraction. The internal elastic lamina separates the tunica intima from the tunica media. Smooth muscle cells (SMC) and extracellular matrix (ECM) proteins make up the tunica media and are the contractile force in maintaining hemodynamics and tissue perfusion. Fibroblasts are the major cell type of the adventitia and are supported by ECM proteins. Pathological vascular remodeling can occur in response to a variety of vascular insults such as mechanical (stents or vessel grafts) or biological (hypertension, lipids, smoking) injuries. Changes in the cellular and non-cellular components of the vessel alter the architecture of the vessel through thickening or thinning of various layers. This often produces a reduced vessel lumen diameter, called inward remodeling, or it can also increase lumen diameter in outward remodeling [1].

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Vascular remodeling is a polygenic process and involves multiple cell types from the vessel wall and from circulation such as ECs, SMCs, fibroblasts, leukocytes and platelets. Endothelial damage or dysfunction is believed to be an important initial trigger of subsequent vascular wall structural changes. Normal ECs synthesize and secrete biological substances critical for normal vascular function and structural integrity. For example, EC-released prostanoid prostacyclins (such as PGI₂) or nitric oxide (NO) not only function as a vasodilator, but also inhibit SMC proliferation and migration as well as platelet activation and adhesion, leukocyte adhesion and infiltration, all key events associated with pathological remodeling [2]. Another critical event is SMC phenotypic modulation. In a normal mature blood vessel, SMCs exhibit a contractile or differentiation phenotype, and function principally to maintain vascular tone [3,4]. However, vascular SMCs retain plasticity to undergo phenotypic modulation. In response to vascular injuries and endothelial dysfunction, SMCs change from a contractile, differentiated phenotype to a myofibroblast-like synthetic phenotype [5]. Synthetic SMCs acquire the capacity to proliferate, migrate, take up lipids, and secrete inflammatory molecules and ECM proteins. In the adventitial layer, fibroblasts can transdifferentiate into myofibroblasts, which can produce ECM proteins that contribute to vessel thickening [6]. Circulating monocytes, which differentiate into macrophages, and other leukocytes play vital roles in the initiation and progression of vascular lesions. Under pathological conditions, vascular and infiltrating cells release cytokines, chemokines and adhesion molecules that facilitate immune cell adhesion, infiltration and activation [3,7–9].

Cyclic nucleotides (cAMP and cGMP) serve as secondary messenger molecules and are crucial regulators of vascular structural integrity and function. PDEs, by catalyzing the hydrolysis of cyclic nucleotides, play very important roles in the regulation of the amplitude, duration, and compartmentalization of intracellular cyclic nucleotide signaling. Alterations of PDE expression or activity can disrupt the homeostasis of cyclic nucleotides, contributing to disease progression. PDE inhibitors may be effective to treat the diseases.

Cyclic nucleotides and PDEs

cAMP and cGMP are formed from corresponding nucleotide monophosphates by adenylyl and guanylyl cyclases, respectively, and degraded by PDEs. In normal vascular tissue, cAMP is a critical mediator of β -adrenergic and prostacyclin signaling, and cGMP is a mediator of signaling through NO and natriuretic peptides such as ANP and CNP. cAMP-dependent protein kinase (PKA) or cGMP-dependent protein kinase (PKG) are the primary effector molecules of cAMP and cGMP, respectively. It is well known that both PKA and PKG activation leads to vasorelaxation by lowering intracellular Ca²⁺ concentrations and promoting myosin light chain dephosphorylation [10]. cAMP or cGMP can also directly interact with other molecules. For example, cAMP can activate exchange proteins activated by cAMP (Epac), guanine nucleotide exchange factors for small GTPase Rap1 and Rap2. Epac may be involved in various cellular processes involved in vascular remodeling, such as smooth muscle proliferation, leukocyte migration and endothelial barrier function [11,12]. Additionally, cAMP and cGMP can activate cyclic nucleotide-gated cation channels (CNG), leading to intracellular Ca²⁺ elevation [13]. Although the knowledge of CNG is largely restricted to sensory neurons, recent evidence suggests that CNG is also expressed in non-sensory cells, including vascular cells [14–16]. Moreover cGMP may regulate cAMP signaling through modulating cGMP stimulated or inhibited PDEs [17,18].

PDEs are a superfamily of phosphohydrolases that catalyze the degradation of cAMP and cGMP to inactive 5'-AMP and 5'-GMP respectively. To date, there are over 60 PDE isoenzymes derived from 21 genes. The gene products are grouped into eleven broad families, PDE1–PDE11, based on their distinct kinetic properties, regulatory mechanisms

and sensitivity to selective inhibitors [19]. In the vascular system, PDE1–5 are known to be involved in many functions of vascular cells; however, the expression and function of PDE7–11 have not been well characterized in the cardiovascular system. This review article will focus on PDE1 in vascular biology and disease.

The PDE1 family is a large family encoded by three distinct genes, PDE1A, 1B and 1C with multiple N- or C- terminal splice variants within those isoforms [19]. *In vitro*, PDE1 catalytic activity can be stimulated by calcium and calmodulin to increase basal activity up to 10-fold or more. This is the reason PDE1 is also referred to as Ca^{2+} /calmodulin-stimulated PDE [20]. Ca^{2+} -dependent activation of PDE1 isozymes may play a role in crosstalk between Ca^{2+} mediated pro-remodeling signaling and cyclic nucleotide-mediated anti-remodeling. Indeed, it has been shown that Ca^{2+} -mediated reduction of cGMP can be recovered by PDE1 inhibitors [21–23]. Different PDE1 isozymes differ in their regulatory properties, substrate affinity, Ca^{2+} sensitivity and tissue/cell distribution. *In vitro*, PDE1A and PDE1B have been shown to have a high specificity for cGMP, while PDE1C is selective for both cGMP and cAMP. *In vivo*, PDE1A has been shown to primarily regulate cGMP in cardiomyocytes and vascular SMC [24,25]. PDE1B has also been found to regulate intracellular cGMP levels in macrophage-derived cell line HL-60 [26]. PDE1C has been shown to regulate cAMP in various cell types including aortic SMC [27], pulmonary artery SMCs [28], pancreatic β -cells [29], and glioblastoma cells [30]. It has not been reported that PDE1C regulates intracellular cGMP.

PDE1 in Pathological Vascular Remodeling and Disease

The studies of PDE1 in vasculature have focused primarily on vascular SMCs and myofibroblast-like cells. This is because in the vasculature, PDE1 activity is primarily present in SMCs. PDE1 is not evident in ECs [31–33], although there might be some controversial reports of PDE1 in EC [34,35]. Using PDE1 inhibitors, it has been shown that PDE1 activity is critical for synthetic SMC growth, survival and ECM synthesis. More importantly, inhibition of PDE1 activity attenuates human saphenous vein remodeling [16•]. These observations strongly suggest that PDE1 activity is critical in pathological vascular remodeling. Different PDE1 isoforms are expressed and regulated differentially and play distinctive roles in regulating SMC function and vascular remodeling.

PDE1A

PDE1A has been detected in both contractile and synthetic SMCs [25]. However, subcellular PDE1A location varies depending on the state of SMC in the vessel [25]. In contractile SMCs, PDE1A was shown to be located mainly in the cytosol, while PDE1A is located in the nucleus in synthetic SMCs [25]. The cytosolic PDE1A of contractile SMCs appears to regulate myosin light chain phosphorylation, suggesting the role of PDE1A in regulating SMC contractility [25]. Indeed, it has been shown that PDE1A expression and activity is upregulated in a nitrate tolerance animal model, in which NO-mediated vasodilation is impaired [21]. Vinpocetine, used as a PDE1 inhibitor, largely reversed the nitrate tolerance, suggesting that PDE1A is important in regulating vascular reactivity [21]. The role of PDE1A in vascular contraction is further supported by the recent study showing that PDE1A upregulation is likely responsible for the increased contractile responsiveness of small mesenteric arteries in angiotensin II (Ang II)-infused rats [36•]. The nuclear PDE1A, however, has unique functions in synthetic SMCs. It has been shown that nuclear PDE1A has limited effects on myosin light chain phosphorylation but is critical for synthetic SMC cell growth and survival [25]. Inhibiting PDE1A activity by general PDE1 inhibitor IC86340 or specific PDE1A shRNA drastically inhibits cell cycle progression and promotes cell apoptosis of synthetic SMCs [25]. In addition, nuclear PDE1A regulates nuclear β -catenin protein stability through the nuclear PP2A–GSK3 β – β -catenin signaling axis [37•]. These

suggest that nuclear PDE1A promotes cell growth through enhancing the β -catenin/T-cell factor (TCF) pathway.

During vascular remodeling, adventitial fibroblasts are activated by differentiating into myofibroblasts. Adventitial myofibroblasts are very similar to synthetic SMCs (also often referred to myofibroblasts). The expression of PDE1A in adventitial myofibroblasts appears to be similar to synthetic SMCs. For example, PDE1A expression levels are very low in normal adventitial fibroblasts, but TGF β stimulation of fibroblast differentiation significantly upregulates nuclear PDE1 expression [38•]. TGF β also stimulates the expression of alpha smooth muscle actin (α -SMA), a marker of myofibroblast differentiation. Induction of PDE1A plays a critical role in α -SMA induction, through regulating PKC α important in myofibroblast differentiation [38•].

PDE1B

The reports of PDE1B in the vascular cells are limited. PDE1B is not detected in rodent and human SMCs, but detected in cultured monkey SMC [27]. Although PDE1B may not be a major PDE1 isoform in vascular cells, PDE1B in macrophages may contribute to pathological vascular remodeling. It has been shown that PDE1B, not PDE1A or 1C, is specifically upregulated during monocyte to macrophage differentiation induced by granulocyte macrophage colony stimulating factor (GM-CSF)[39]. Although the precise role of PDE1B in macrophages is still unknown, PDE1B in vascular remodeling deserves to be determined *in vitro* and *in vivo*.

PDE1C

PDE1C was reported to be induced in growing aortic SMCs of human fetal aorta, human vascular lesions, and cultured human SMCs, but not in normal human vessels [27]. This suggests that PDE1C is specifically associated with synthetic SMCs. In cultured human aortic SMCs, PDE1C promotes cell proliferation [40]. Because these previous studies failed to detect PDE1C expression in growing rodent SMCs, it has long been thought that PDE1C is only expressed in SMCs from human but not other species. However, we recently found that PDE1C is also expressed in rodent SMCs when these cells are prepared and cultured under appropriate culture conditions [16•]. Thus induction of PDE1C in synthetic SMCs appears to be conserved among different species. Although both PDE1A and PDE1C regulate SMC proliferation, the underlying mechanisms are likely different. For example, PDE1A but not PDE1C regulates the β -catenin/TCF signaling pathway, which is known to be critical in cell growth [37•]. In addition to cell proliferation, PDE1C also plays a critical role in regulating type I collagen in synthetic SMCs through a mechanism involving lysosome-dependent collagen degradation [16•].

PDE1 and Pulmonary Hypertension

Pulmonary hypertension (PH) is a vascular disease that involves arterial vessel dysfunction and remodeling causing elevated pulmonary artery pressure and reduced lung perfusion [41]. PDE1 isoforms are found in pulmonary artery SMCs and may play critical roles in regulating pulmonary vascular hemodynamics and remodeling. For example, increased cGMP-hydrolyzing PDE1 activity was reported in main pulmonary artery of hypoxia-induced PH rats [42]. In a rat pulmonary vascular remodeling model induced by sinoaortic denervation, PDE1A and PDE1C expression levels were significantly upregulated, accompanied with decreased tissue cGMP [43]. Moreover, an increase of PDE1A and 1C expression along with other PDE family members was found in human pulmonary artery SMCs from patients with either idiopathic PH or secondary PH [28]. PDE1C-targeted siRNA enhanced cAMP accumulation and inhibited cellular proliferation, suggesting that an

increase of PDE1C contributes to decreased cAMP and increased proliferation of pulmonary artery SMCs in PH patients [28]. In various animal PH models, investigators were able to improve hemodynamics in the pulmonary circuit and reduce smooth muscle cell proliferation using PDE1 inhibitors either by itself or in combination with other therapies such as prostacyclin analogues and nitric oxide [44–46]. These imply that PDE1 isoforms may provide novel therapeutic targets for the treatment of PH.

Conclusion and Perspective

To date, most information of PDE1 isoforms in the cardiovascular system is derived from *in vitro* studies with cultured SMCs. The *in vivo* functional studies are limited due to lack of isoform specific inhibitors or genetically manipulated transgenic or knockout mice. Among the PDE1 isozymes, PDE1A is expressed in the cytoplasm of normal contractile SMCs as well as in the nuclei of synthetic SMCs or adventitial myofibroblasts [25,38•]. Although the molecular identities of the cytosolic and nuclear PDE1A are still not known, PDE1A in these two compartments may play distinctive roles, with cytosolic PDE1A for regulating contractile function and nuclear PDE1A for cell growth/survival [25]. Thus, a PDE1A-specific inhibitor might be useful for treating hypertension as well as vascular diseases associated with pathological vascular remodeling. Because hypertension and pathological vascular remodeling often happen concurrently with aging population, PDE1A inhibitors might be beneficial to older adults.

The regulation and function of PDE1C in vascular SMCs are different from PDE1A. PDE1C in the vasculature appears to be exclusively functional in growing SMCs during the normal vascular development and pathological vascular remodeling [27]. The fact that PDE1C is only in synthetic SMCs of disease vessels but not in the normal vasculature suggests that a PDE1C-specific inhibitor will only target disease processes and not interfere with normal vascular function.

Although there is no PDE1 isoform-specific inhibitor reported yet, several general PDE1 inhibitors have been used experimentally. For example, vinpocetine was used as a PDE1 inhibitor in many *in vivo* animal studies [21,47–49]. Because vinpocetine has several non-PDE1 targets including Ca²⁺ channels [50], Na⁺ channels [50], and IκB kinase (IKK) [51], all of which regulate vascular function and structural remodeling, the vinpocetine effects seen may not mediated by PDE1 inhibition. 8-MM-IBMX was also used in several *in vivo* animal studies [28]. However, 8-MM-IBMX concentrations reached *in vivo* were not carefully evaluated in these studies. It is known that 8-MM-IBMX at high concentrations also inhibit many other PDEs [30,40]. Thus, it is questionable whether the effects of 8-MM-IBMX are mainly through PDE1 inhibition. Recently, we have used IC86340 (initially from ICOS Inc) [24,25]. IC86340 has much higher selectivity for PDE1 over other PDE families in the vasculature [25]. Interestingly, a series of PDE1 inhibitors recently developed by Intra-Cellular Therapies, Inc. are in preclinical development for treating schizophrenia [52]. Given that clinically relevant PDE1 inhibitors may be soon available for treating neuronal disorders, it will be of great interest to evaluate the therapeutic effects of these PDE1 inhibitors on cardiovascular diseases.

Highlights

- PDE1 is important in the mechanism of vascular remodeling
- PDE1 isozymes have critical roles in smooth muscle cell dysfunction
- PDE1 may have roles in other cells in vascular remodeling

- PDE1 inhibitors have potential therapies in vascular remodeling

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