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Commentary

Cyclic nucleotide phosphodiesterase 1 and vascular aging

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Abstract

VSMCs (vascular smooth muscle cells) play critical roles in arterial remodelling with aging, hypertension and atherosclerosis. VSMCs exist in diverse phenotypes and exhibit phenotypic plasticity, e.g. changing from a quiescent/contractile phenotype to an active myofibroblast-like, often called 'synthetic', phenotype. Synthetic VSMCs are able to proliferate, migrate and secrete ECM (extracellular matrix) proteinases and ECM proteins. In addition, they produce pro-inflammatory molecules, providing an inflammatory microenvironment for leucocyte penetration, accumulation and activation. The aging VSMCs have also shown changes in cellular phenotype, responsiveness to contracting and relaxing mediators, replicating potential, matrix synthesis, inflammatory mediators and intracellular signalling. VSMC dysfunction plays a key role in age-associated vascular remodelling. Cyclic nucleotide PDEs (phosphodiesterases), by catalysing cyclic nucleotide hydrolysis, play a critical role in regulating the amplitude, duration and compartmentalization of cyclic nucleotide signalling. Abnormal alterations of PDEs and subsequent changes in cyclic nucleotide homoeostasis have been implicated in a number of different diseases. In the study published in the latest issue of *Clinical Science*, Bautista Niño and colleagues have shown that, in cultured senescent human VSMCs, *PDE1A* and *PDE1C* mRNA levels are significantly up-regulated and inhibition of PDE1 activity with vinpocetine reduced cellular senescent makers in senescent VSMCs. Moreover, in the premature aging mice with genomic instability (*Ercc1*$^{d/-}$), impaired aortic ring relaxation in response to SNP (sodium nitroprusside), an NO (nitric oxide) donor, was also largely improved by vinpocetine. More interestingly, using data from human GWAS (genome-wide association studies), it has been found that *PDE1A* single nucleotide polymorphisms is significantly associated with diastolic blood pressure and carotid intima–media thickening, two hallmarks of human vascular dysfunction in aging. These findings establish a strong relationship between PDE1 expression regulation and vascular abnormalities in aging.

Key words: aging, cyclic nucleotide phosphodiesterase, extracellular matrix, genome-wide association study, intima–media thickening, vascular smooth muscle cells.

CVD (cardiovascular disease) remains the leading cause of morbidity and mortality in the aging population. VSMCs (vascular smooth muscle cells) play critical roles in arterial remodelling with aging, hypertension and atherosclerosis. Under normal conditions, VSMCs residing in the media of vessels are quiescent with a very low turnover rate and insignificant secretory activity. These VSMCs are highly differentiated cells that possess the contractile phenotype by expressing large amounts of contractile proteins, and function principally to maintain vascular tone. VSMCs exist in diverse phenotypes and exhibit phenotypic plasticity, e.g. changing from a quiescent/contractile phenotype to an active myofibroblast-like, often called 'synthetic', phenotype [1]. Synthetic VSMCs are able to proliferate, migrate and secrete ECM (extracellular matrix) proteinases and other ECM proteins. In addition, they produce pro-inflammatory molecules, providing an inflammatory microenvironment for leucocyte penetration, accumulation and activation [2,3]. The aging VSMCs have also shown changes in cellular phenotype, responsiveness to contracting and relaxing mediators, replicating potential, matrix synthesis, inflammatory mediators and intracellular signalling [4,5]. VSMC dysfunction plays a key role in age-associated vascular remodelling.

Cyclic nucleotide PDEs (phosphodiesterases), by catalysing cyclic nucleotide hydrolysis, play a critical role in regulating the amplitude, duration and compartmentalization of cyclic nucleotide signalling. PDEs constitute a superfamily of enzymes with 22 different genes and more than 100 different mRNAs grouped into 11 broad families (PDE1–PDE11) on the basis of distinct

Abbreviations: AngII, angiotensin II; CaM, calmodulin; ECM, extracellular matrix; GWAS, genome-wide association studies; NF-κB, nuclear factor κB; NTG, nitroglycerin; PDE, phosphodiesterase; SMC, smooth muscle cell; VSMC, vascular smooth muscle cell.

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Table 1 PDE1 family members
CaM, calmodulin; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; PDE, phosphodiesterase; PKA, protein kinase A.

PDE1 gene	Variants	Substrates (K_m) [7]	Inhibitors (IC_{50}) [7,14]	Regulators [7,36]	Expression in VSMCs [14–16]	Roles in VSMCs [14–16]	Association with vascular diseases [16,27]
PDE1A	1A1–1A14	cAMP: ~112 μM; cGMP: ~5 μM	Vinpocetine: ~10 μM; IC86340: ~0.4 μM	Ca^{2+}/CaM; PKA	Contractile: cytosol; synthetic: nucleus	Contraction, growth, death	Increased in nitrate tolerance; increased in premature aging mice (*Ercc1*$^{d/-}$); single nucleotide polymorphism association with high blood pressure and intima–media thickening in humans
PDE1B	1B1–1B2	cAMP: ~24 μM; cGMP: ~3 μM	Vinpocetine: ~10 μM; IC86340: ~0.2 μM	Ca^{2+}/CaM; CaMKII	Only detected in VSMCs of monkeys and baboons	Unknown	Unknown
PDE1C	1C1–1C5	cAMP: ~1 μM; cGMP: ~1 μM	Vinpocetine: ~40 μM; IC86340: ~0.06 μM	Ca^{2+}/CaM	Contractile: none; synthetic: membrane and cytosol	Growth, migration, collagen	Induced in neointimal VSMCs of rodent and human vessels; increased in premature aging mice (*Ercc1*$^{d/-}$)

structural, kinetic, regulatory and inhibitory properties. PDEs are expressed in a cell/tissue-specific manner with only a few enzymes expressed in any single cell type. PDE1 family members are Ca^{2+}/CaM (calmodulin)-stimulated PDEs encoded by three distinct genes, *PDE1A*, *PDE1B* and *PDE1C* (Table 1). The enzyme activity of all PDE1 isoenzymes can be stimulated up to 10-fold by Ca^{2+}/CaM *in vitro* as well as be modulated by other kinases [6]. PDE1A and PDE1B isoenzymes hydrolyse cGMP with much higher affinities than cAMP, whereas PDE1C isoenzymes hydrolyse both cAMP and cGMP with equally high affinity [7]. The unique Ca^{2+}-stimulated property allows PDE1 members to function as important mediators in the cross-talk between Ca^{2+} and cyclic nucleotide signalling [8]. In the vasculature, PDE1 activity is primarily associated with VSMCs, but not endothelial cells [9,10]. PDE1A is found in large vessels from many different species [11–13] as well as in cultured VSMCs [14]. PDE1B is only reported in VSMCs from monkeys and baboons [13,15]. PDE1C is detected in growing VSMCs in culture and in vascular lesions, but not in normal medial VSMCs [15,16]. These findings suggest that PDE1 expression is species- and cell-phenotype-dependent.

Abnormal alterations of PDEs and subsequent changes in cyclic nucleotide homoeostasis have been implicated in a number of different diseases [17]. In the study published in the latest issue of *Clinical Science*, Bautista Niño et al. [18] have shown that, in cultured senescent human VSMCs, *PDE1A* and *PDE1C* mRNA levels are up-regulated 11.6- and 9-fold respectively. Inhibition of PDE1 activity with vinpocetine reduced cellular senescent makers (such as p16 and p21) in senescent VSMCs. Moreover, in the premature aging mice with genomic instability (*Ercc1*$^{d/-}$), impaired aortic ring relaxation in response to SNP (sodium nitroprusside), an NO (nitric oxide) donor was also largely improved by vinpocetine. More interestingly, using data from human GWAS (genome-wide association studies), it has been found that *PDE1A* single nucleotide polymorphisms is significantly associated with diastolic blood pressure and carotid intima–media thickening, two hallmarks of human vascular dysfunction in aging. These findings establish a strong relationship between the regulation of PDE1 expression and vascular abnormalities in aging. Although it was attempted to determine the potential roles for PDE1 in vascular aging using vinpocetine as a PDE1 inhibitor, the observed vinpocetine effects may not be entirely mediated by PDE1 inhibition. This is because vinpocetine has several non-PDE1 targets, including Ca^{2+} channels [19], IKK [inhibitor of NF-κB (nuclear factor κB) kinase]/NF-κB [20], ROS (reactive oxidative species) production [21] and MKP-1 (mitogen-activated protein kinase phosphatase-1) [22], all of which may be also important in vascular senescence and dysfunction. Other previous studies of PDE1A and PDE1C in cultured VSMCs and animal models may also provide supportive evidence.

The potential role of PDE1 in regulating vascular reactivity and blood pressure has been implicated from a number of previous studies. It has been shown that vinpocetine increases cGMP levels, accompanied by dilating rabbit and rat aortas in *ex vivo* organ culture [23–27]. These data suggest that PDE1 is important in regulating cGMP signalling and smooth muscle relaxation. Most vasoconstrictors, such as noradrenaline (norepinephrine), AngII (angiotensin II) and ET-1 (endothelin 1), increase intracellular Ca^{2+}, which is thought to be the major mechanism of vasoconstrictor-mediated smooth muscle contraction. cGMP functions as a negative regulator of intracellular Ca^{2+} elevation

and vasoconstriction [8]. It is therefore logical that vasoconstrictors increase the activity of PDE1 via increased Ca^{2+}, which then decreases cGMP levels and promotes vasoconstriction. Indeed, it has been shown that PDE1 activity is rapidly stimulated in rabbit arterial strips and in cultured rat aortic VSMCs by Ca^{2+}-elevating reagents such as AngII [27–29], which is responsible for AngII-mediated antagonism of ANP (atrial natriuretic peptide)-induced cGMP accumulation [27]. The PDE1 isoenzyme is preferentially PDE1A because PDE1B and PDE1C expression is negligible in medial contractile VSMCs [15,16], and PDE1C deficient mice do not have blood pressure changes [16]. In a rat nitrate tolerance model induced by continuous NTG (nitroglycerin), an NO donor, infusion for 3 days, increased PDE1A expression and activity was found in the tolerant rat aortas [27]. Vinpocetine partially restored the vasodilatory sensitivity of tolerant vessels to subsequent NTG exposure. It has been shown that a more specific PDE1 inhibitor, IC86340, reduces basal blood pressure (∼10–20 mmHg) in mice [30]. Collectively, these experimental results suggest that PDE1A is important in regulating vascular reactivity, which is consistent with the human GWAS showing PDE1A association with blood pressure dysregulation reported in a previous study [31] as well as in the study by Bautista Niño et al. [18]. Owing to lack of a PDE1A-selective inhibitor, genetically engineered PDE1A mice will be useful to understand the function and underlying mechanism of the PDE1A isoenzyme in blood pressure regulation under normal and disease states.

The regulation and function of PDE1 in VSMC proliferation/migration and intima–media thickening has also been previously investigated *in vitro* and *in vivo*. PDE1A localizations appear to be VSMC phenotype-dependent. For example, it has been shown that in VSMCs with the contractile phenotype both *in vitro* and *in vivo*, PDE1A is predominantly cytoplasmic. In contrast, PDE1A is expressed in the nucleus of synthetic VSMCs in culture or neointimal lesions [14]. Cytoplasmic PDE1A appears to control the contractility of contractile VSMCs, whereas nuclear PDE1A is critical for synthetic VSMC growth and survival [14]. PDE1A promotes VSMC proliferation through increasing the protein stability of nuclear β-catenin, an essential transcription modulator in cell proliferation [32]. These findings are consistent with the GWAS showing a significant association of *PDE1A* single nucleotide polymorphisms with carotid intima–media thickness [31]. Experimental evidence has also revealed an important role for PDE1C in pathological vascular remodelling. It has been shown that PDE1C expression is almost not present in contractile SMCs (smooth muscle cells), but drastically elevated in synthetic SMCs *in vitro* as well as in neointimal lesions of animal models and human disease vessels [15,16]. PDE1C knockout or PDE1-selective inhibitor significantly attenuates VSMC growth/migration *in vitro* and injury-induced neointimal formation *in vivo* [16,33]. More importantly, PDE1 inhibition suppressed vascular remodelling of human saphenous vein explants in an *ex vivo* organ culture model [16]. Mechanistic studies revealed that PDE1C inhibits endocytosis/lysosome-dependent degradation and thus increases the protein stability of growth factor receptors, such as PDGFRβ (platelet-derived growth factor receptor β) known to be important in pathological vascular remodelling [16]. In addition, PDE1C has also been shown to be critical in regulating collagen homoeostasis by inhibiting lysosome-dependent type I collagen protein degradation and thus increasing collagen production in synthetic VSMCs [34]. PDE1 inhibitors may represent novel therapeutic agents for treating cardiovascular diseases.

Taken together, the findings from previous studies and that of Bautista Niño [18] strongly support the conclusion that PDE1A and PDE1C induction/activation may play key roles in VSMC pathogenesis associated with abnormal vascular reactivity and intima–media thickening, via different molecular mechanisms. In addition, the experimental evidence is also in line with the human GWAS showing PDE1A association with blood pressure dysregulation and intima–media thickening reported by Bautista Niño et al. [18]. Future studies are necessary to determine the causative roles and underlying mechanisms of PDE1A and PDE1C in vascular aging using various *in vitro* and *in vivo* approaches. Owing to the lack of PDE1 isoenzyme-selective inhibitors, developing genetically engineered aging mice with gain- or loss-of-PDE1A or -PDE1C function will be useful. In addition, given the fact that PDE1A and PDE1C are differentially expressed and function in contractile and synthetic SMCs, a combination of targeting both PDE1A and PDE1C may have additive or synergistic effects in treating vascular disorders. A series of pan-PDE1 inhibitors, recently developed by Intra-Cellular Therapies, Inc., are in pre-clinical development for treating schizophrenia [35]. Thus PDE1 inhibitors may represent feasible therapeutic agents for treating cardiovascular diseases associated with aging.

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