Arteriosclerosis, Thrombosis, and Vascular Biology

EDITORIAL

Natriuretic Peptides and the Regulation of Retinal Neovascularization

Denise Burtenshaw, Paul A. Cahill

The NP (natriuretic peptide) family includes ANP (atrial NP), BNP (brain NP), and CNP (C-type NP) that bind 3 membrane-bound receptors. The NPR-A (NP receptor-A or guanylyl cyclase-A) and the NPR-B (NP receptor-B or guanylyl cyclase-B) both increase cyclic GMP (cGMP) to activate PKG (cGMP-dependent protein kinase) and stimulate downstream effectors involved in cell growth, apoptosis, proliferation, and inflammation.[1] The NPR-C (NP receptor-C or clearance receptor) is coupled to a Gi (inhibitory G protein) and stimulates ERK (extracellular signal-regulated kinase)-1/2 and Akt (protein kinase B) to promote endothelial proliferation, migration, and survival.[1] Over the last decade, numerous studies have provided compelling evidence for a putative role of these 3 structurally similar natriuretic hormones in controlling vascular function.[2] ANP and BNP are predominantly secreted into the circulation from cardiomyocytes in response to stretching of the myocardium. These peptides induce a decrease in vascular tone, an immediate increase in electrolyte and water excretion via the kidney, and antifibrotic and antihypertrophic effects in the heart, all of which functionally antagonize the renin-angiotensin-aldosterone system.[3] In contrast, endothelium-derived CNP plays a pivotal role in angiogenesis and vascular remodeling after ischemia via specific activation of NPR-C, Gi stimulation, and ERK1/2 and Akt phosphorylation.[4] The presence of all 3 hormones and their cognate receptors within the human retina is thought to facilitate a local system of NP regulation of neural and vascular components to maintain both nerve and vessel integrity.[5,6]

See accompanying article on page 159

Pathological blood vessel formation (neovascularization) within the retina poses a significant threat to normal vision. Angiogenesis, essential for both physiological vascular development and pathological neovascularization, occurs as endothelial cells proliferate and form new blood vessels in response to guidance cues and angiogenic factors/inhibitors. Pathological angiogenesis is associated with many diseases, including cancer, cardiovascular disease, neurodegeneration, and proliferative retinopathies.[7] Within the eye, neovascularization is characterized by leaky tuft-like vessels and associated with retinal exudates and hemorrhage, culminating in retinal damage and detachment.[8] It occurs in a broad spectrum of eye disorders, such as retinopathy of prematurity, proliferative diabetic retinopathy (PDR), neovascular age-related macular degeneration, neovascular glaucoma, and corneal neovascularization.[9] The pathophysiological similarities between PDR and retinopathy of prematurity suggest that progressive vessel loss as a result of increased oxygen may underlie the subsequent hypersecretion of VEGF (vascular endothelial growth factor). Moreover, these changes are associated with inflammatory infiltration and formation of neovascular tufts leading to visual impairment.[10,11] While a role of the NPR-A system in the stimulation of postischemic neovascularization following cardiac hypertrophy and hindlimb ischemia has been reported,[12] the definitive role of specific NP receptors as stress-responsive regulators of angiogenesis and neovascularization in the retina remains unclear.

The recent publication by Spes et al[13] in this issue of *ATVB* addresses the specific role of ANP/BNP in dictating physiological and pathophysiological neovascularization typical of PDR and retinopathy of prematurity phenotypes. While a role for NPs as potent modulators of VEGF-induced vascular leakage and angiogenesis in

Key Words: Editorials ■ atrial natriuretic factor ■ humans ■ retina ■ vascular remodeling ■ water

Correspondence to: Paul A. Cahill, BSc, PhD, Vascular Biology and Therapeutics, Faculty of Science and Health, School of Biotechnology, Dublin City University, Lonsdale Bldg, Office X2-09, Glasnevin, Dublin 9, Ireland. Email paul.cahill@dcu.ie

For Disclosures, see page 10.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

vivo is clear,[14] the target cells that mediated this response remain unknown. This study is the first to demonstrate that endogenous NPs exert a protective effect on the retinal vasculature primarily through retinal pericytes. This effect significantly improves developmental vascularization and attenuates pathological ischemia-driven ocular neovascularization. Using oxygen-induced retinopathy as a model of proliferative retinopathy, the authors demonstrate enhanced pathological neovascularization following the global deletion of the NPR-A receptor.[13] The authors then used transgenic mice with restricted deletions of NPR-A in either endothelial cells, pericytes, or astrocytes using floxed NPR-A mice interbred with Tie2-Cre (tyrosine-protein kinase receptor-driven Cre recombinase), GFAP-Cre (glial fibrillary acidic protein-driven Cre recombinase), or PDGF-Rβ-CreERT2 (platelet-derived growth factor beta receptor-driven tamoxifen-dependent Cre recombinases) lines, respectively, to reveal further the various cell types involved. Parallel in vitro studies on cultured pericytes and astrocytes from these animals revealed the potential mechanism(s) involved.

In murine hypertrophic heart and hindlimb models of neovascularization, NPs (in particular, BNP) produced by activated satellite cells regulate the regeneration of neighboring endothelial cells via NPR-A. This paracrine communication was thought critical in coordinating muscle regeneration/hypertrophy and angiogenesis.[12] Surprisingly, in the current study, specific NPR-A deletion in retinal endothelial cells failed to impact retinal vascular development and pathological neovascularization. Similarly, in mice lacking NPR-A signaling in retinal astrocytes, vascular development and hyperoxia-driven vascular regression remained unaltered with ischemia-induced neovascularization modestly increased.[13] In contrast, when the NPR-A was deleted in pericytes, physiological retinal vascularization was reduced concomitant with enhanced pericyte cell apoptosis, vascular regression, and subsequent neovascularization.[13] Follow-up in vitro studies revealed that NP/NPR-A/cGMP signaling also inhibited TGF-β1 (transforming growth factor-β1)–induced pericyte loss and hypoxia-induced VEGF secretion from astrocytes.[13] Collectively, these data reaffirm the putative role of NPs in regulating retinal microvasculature following oxygen-induced retinopathy. As a similar process prevails in several proliferative retinopathies, augmentation of endogenous NP/NPR-A/cGMP signaling may represent a novel therapeutic target for combating retinopathies associated with neovascularization.

Several different functions assigned to pericytes include (1) angiogenesis and vessel stabilization, (2) blood flow regulation and neurovascular coupling, and (3) regulation of the blood-brain barrier and blood-retinal barrier.[15] They also possess a multipotent differentiation capacity akin to mesenchymal stem cells. However, several reports using lineage tracing analysis demonstrate that they may not behave as tissue-specific progenitors in various organs, despite showing mesenchymal stem cell potential in vitro.[15] The evidence that NPs regulate retinal (neo)angiogenesis is compelling.[13,14,16] During early retinal disease, pericyte apoptosis is a major contributor to microvascular endothelial dysfunction and leakage.[17] Hypoxia then drives VEGF secretion from astrocytes and pathological neoangiogenesis.[18] The NPR-A/cGMP receptor is expressed in both vascular and perivascular cell types of the retina (such as endothelial cells, pericytes, and astrocytes), but the functional significance is unknown until now. While NPs, via cGMP, have cytoprotective actions in various types of cells, no reports reveal whether and how these hormones may modulate pericyte viability. In the current study, Spes et al report that NPs, via NPR-A, enhance cGMP levels and cGKI (cGMP-dependent protein kinase I) activity in cultured pericytes. This leads to the phosphorylation of Akt and the expression of the antiapoptotic protein Bcl-2 (B-cell lymphoma 2).[13] Bcl-2 is a well-defined mechanism of survival for many cell types and known to reduce the level of cleaved caspase 3 and apoptosis.[19] It is notable that in the current model of hyperoxia-driven retinal cell apoptosis, loss of pericytes, vascular regression, and enhanced formation of neovascular tufts were all significantly enhanced in pericyte GC-A (guanylyl cyclase A) knockout mice and following global NPR-A deletion.[13]

The proposed model is that NPR-A/cGMP signaling exerts local protective effects through inhibition of stress-induced pericyte apoptosis and reduction of the hypoxic induction of VEGF in astrocytes (Figure). While the source of the specific NPs (ANP or BNP) was not addressed, it is clear that endogenous NPs attenuate vascular regression in murine models of proliferative retinopathy.[13,14,16] Whether the antiapoptotic effects of NPs in cultured pericytes in vitro are mirrored in NPR-A knockout mice in vivo remains unclear. Moreover, the fate of additional cell type(s) due to apoptosis in NPR-A knockout mice remains unresolved.

Despite the compelling evidence for a significant role of the NPR-A receptor in protecting pericytes from apoptosis under pathological conditions, the potential role of the NPR-C or CNP cannot be ruled out. Indeed, endothelial cells, astrocytes, and pericytes all express the NPR-C, which is fundamental to endothelial signaling.[4] Previous studies have reported significant in vitro angiogenic activity of CNP in pulmonary microvascular endothelial cells, human umbilical vein endothelial cells, and aortic rings isolated from wild-type, endothelium-specific CNP$^{-/-}$, the global NPR-B$^{-/-}$, and NPR-C$^{-/-}$ transgenic mice. The proangiogenic effect of CNP/NPR-C is dependent on activation of Gi, ERK1/2, and phosphoinositide 3-kinase-γ/Akt at a molecular level.[4] Indeed, vascular ischemia is associated with reduced levels of CNP and its cognate NPR-C receptor.[20] Genetic or pharmacological inhibition of CNP and NPR-C, but not NPR-B, reduces the angiogenic potential of pulmonary microvascular endothelial cells, human umbilical vein

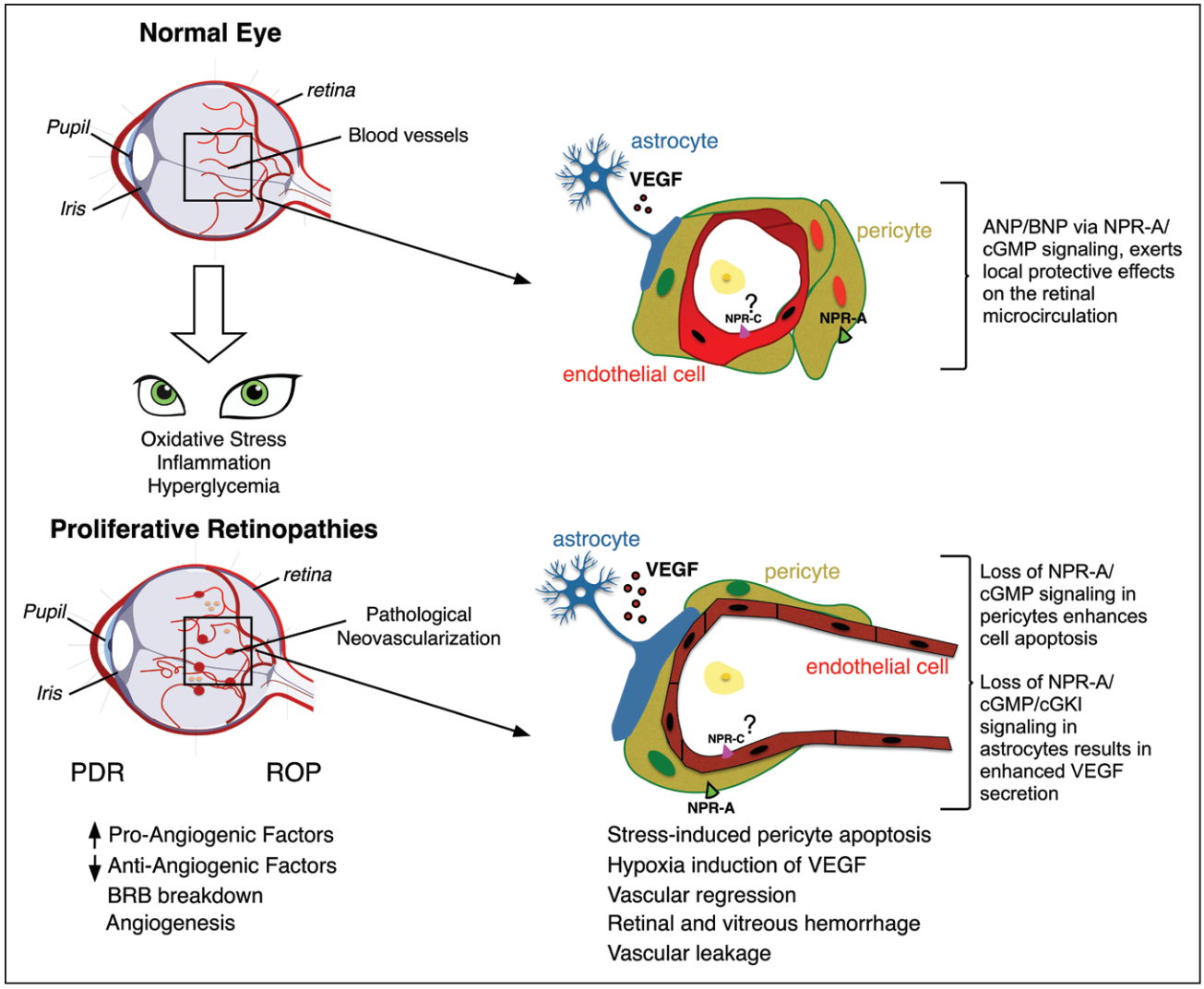

Figure. Schematic showing pathological retinal neovascularization typical of proliferative diabetic retinopathy (PDR) and retinopathy of prematurity (ROP).

Oxidative stress and inflammation lead to accelerated death of retinal microvascular cells (pericytes and endothelial cells). This leads to development of acellular capillaries and retinal ischemia that triggers upregulation of VEGF (vascular endothelial growth factor) expression leading to retinal neovascularization. Natriuretic peptides, via NPR-A (natriuretic peptide receptor-A) cyclic GMP (cGMP) signaling, exert local protective effects on the retinal microcirculation through inhibition of stress-evoked pericyte apoptosis and reduction of the hypoxic induction of VEGF in astrocytes.[13] Endogenously formed ANP (atrial natriuretic peptide) or BNP (brain natriuretic peptide) may attenuate vascular regression and subsequent neovascularization in proliferative retinopathy. The clearance natriuretic peptide CNP (C-type natriuretic peptide) has also been implicated in angiogenesis and vascular remodeling after ischemia via specific activation of NPR-C (natriuretic peptide receptor-C), Gi protein stimulation, and triggering of ERK (extracellular signal-regulated kinase)-1/2 and PI3Kγ (phosphoinositide 3-kinase-γ)/Akt (protein kinase B) phosphorylation.[20] BRB indicates blood-retinal barrier; and cGKI, cGMP-dependent protein kinase I.

endothelial cells, and isolated vessels ex vivo. Angiogenesis and remodeling are both impaired in vivo in endothelium-specific CNP$^{-/-}$ and NPR-C$^{-/-}$, but not NPR-B$^{-/-}$, mice. Moreover, this pathological phenotype associated with endothelial CNP$^{-/-}$ mice, but not the NPR-C$^{-/-}$ mice, is rescued by pharmacological administration of CNP.[20] Hence, there may exist an additional central pathophysiological role for CNP and its receptor NPR-C (in addition to ANP/BNP) in retinal neovascularization following oxygen-induced retinopathy that merits examination in mediating some of the retinopathies typical of PDR and retinopathy of prematurity.

In conclusion, it is clear from the study by Spes et al,[13] in this issue of *ATVB*, that under pathological conditions, NP/NPR-A/cGMP signaling protects pericytes from apoptosis and diminishes astrocyte hypersecretion of VEGF, thereby attenuating retinal vascular regression and subsequent neovascularization. It is noteworthy that a pivotal relationship exists between NPR-A and VEGF in the regulation of angiogenesis. VEGF inhibits NP secretion from endothelial cells whereas CNPs (and other NPs) inhibit VEGF production.[16,21] Whether this represents communication between parallel pathways is unclear, but a mechanism

involving TGF-β1 signaling is implicated as both NPs and VEGF have TGF-β1–responsive promoters.[22] This study proposes a novel paradigm that diminished local NP/cGMP signaling participates in the pathophysiology of retinal proliferative retinopathies through pericyte and astrocyte dysfunctionality. Future preclinical studies are required to assess whether significant clinical benefit may arise from augmentation of endogenous ANP/BNP signaling to reduce pericyte loss, vascular regression, and astrocyte VEGF overproduction in early stages of PDR and other forms of proliferative retinopathy.

ARTICLE INFORMATION

Affiliations

From the Vascular Biology and Therapeutics Laboratories, School of Biotechnology, Faculty of Science and Health, Dublin City University, Ireland.

Disclosures

None.

REFERENCES

1. Woodard GE, Rosado JA. Chapter 3 natriuretic peptides in vascular physiology and pathology. *Int Rev Cell Mol Biol*. 2008;268:59–93. doi:10.1016/S1937-6448(08)00803-4
2. Kerkelä R, Ulvila J, Magga J. Natriuretic peptides in the regulation of cardiovascular physiology and metabolic events. *J Am Heart Assoc*. 2015;4:e002423. doi: 10.1161/JAHA.115.002423
3. Nakagawa Y, Nishikimi T, Kuwahara K. Atrial and brain natriuretic peptides: hormones secreted from the heart. *Peptides*. 2019;111:18–25. doi: 10.1016/j.peptides.2018.05.012
4. Moyes AJ, Hobbs AJ. C-type natriuretic peptide: a multifaceted paracrine regulator in the heart and vasculature. *Int J Mol Sci*. 2019;20:2281. doi: 10.3390/ijms20092281.
5. Rollín R, Mediero A, Roldán-Pallarés M, Fernández-Cruz A, Fernández-Durango R. Natriuretic peptide system in the human retina. *Mol Vis*. 2004;10:15–22. Available at http://www.ncbi.nlm.nih.gov/pubmed/14737067. Accessed October 23, 2019.
6. Wolfensberger TJ, Holz FG, Ationu A, Carter ND, Bird AC. Natriuretic peptides and their receptors in human neural retina and retinal pigment epithelium. *Ger J Ophthalmol*. 1994;3:248–252.
7. Grant MB, Lutty GA. Retinal and choroidal vasculature: retinal oxygenation. *Retina*. 2013:433–446. doi:10.1016/B978-1-4557-0737-9.00018-7.
8. Al-Latayfeh M, Silva PS, Sun JK, Aiello LP. Antiangiogenic therapy for ischemic retinopathies. *Cold Spring Harb Perspect Med*. 2012;2:a006411. doi: 10.1101/cshperspect.a006411
9. Sun Y, Smith LEH. Retinal vasculature in development and diseases. *Annu Rev Vis Sci*. 2018;4:101–122. doi: 10.1146/annurev-vision-091517-034018
10. Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. *Ophthalmology*. 2015;122:200–210. doi: 10.1016/j.ophtha.2014.07.050
11. Moran EP, Wang Z, Chen J, Sapieha P, Smith LE, Ma JX. Neurovascular cross talk in diabetic retinopathy: pathophysiological roles and therapeutic implications. *Am J Physiol Heart Circ Physiol*. 2016;311:H738–H749. doi: 10.1152/ajpheart.00005.2016
12. Kuhn M, Völker K, Schwarz K, Carbajo-Lozoya J, Flögel U, Jacoby C, Stypmann J, van Eickels M, Gambaryan S, Hartmann M, et al. The natriuretic peptide/guanylyl cyclase–a system functions as a stress-responsive regulator of angiogenesis in mice. *J Clin Invest*. 2009;119:2019–2030. doi: 10.1172/JCI37430
13. Špiranec Spes K, Hupp S, Werner F, Koch F, Völker K, Krebes L, Kämmerer U, Heinze KG, Braunger BM, Kuhn M. Natriuretic peptides attenuate retinal pathological neovascularization via cyclic guanosine monophosphate signaling in pericytes and astrocytes. *Arterioscler Thromb Vasc Biol*. 2020;40:159–174. doi: 10.1161/ATVBAHA.119.313400
14. Lara-Castillo N, Zandi S, Nakao S, Ito Y, Noda K, She H, Ahmed M, Frimmel S, Ablonczy Z, Hafezi-Moghadam A. Atrial natriuretic peptide reduces vascular leakage and choroidal neovascularization. *Am J Pathol*. 2009;175:2343–2350. doi: 10.2353/ajpath.2009.090439
15. Cano E, Gebala V, Gerhardt H. Pericytes or mesenchymal stem cells: is that the question? *Cell Stem Cell*. 2017;20:296–297. doi: 10.1016/j.stem.2017.02.005
16. Pedram A, Razandi M, Levin ER. Natriuretic peptides suppress vascular endothelial cell growth factor signaling to angiogenesis. *Endocrinology*. 2001;142:1578–1586. doi: 10.1210/endo.142.4.8099
17. Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: key functions and signaling pathways. *Nat Neurosci*. 2016;19:771–783. doi: 10.1038/nn.4288
18. Shortt AJ, Howell K, O'Brien C, McLoughlin P. Chronic systemic hypoxia causes intra-retinal angiogenesis. *J Anat*. 2004;205:349–356. doi: 10.1111/j.0021-8782.2004.00351.x
19. Zhou H, Li XM, Meinkoth J, Pittman RN. Akt regulates cell survival and apoptosis at a postmitochondrial level. *J Cell Biol*. 2000;151:483–494. doi: 10.1083/jcb.151.3.483
20. Bubb KJ, Aubdool AA, Moyes AJ, Lewis S, Drayton JP, Tang O, Mehta V, Zachary IC, Abraham DJ, Tsui J, et al. Endothelial C-type natriuretic peptide is a critical regulator of angiogenesis and vascular remodeling. *Circulation*. 2019;139:1612–1628. doi: 10.1161/CIRCULATIONAHA.118.036344
21. Doi K, Itoh H, Komatsu Y, Igaki T, Chun TH, Takaya K, Yamashita J, Inoue M, Yoshimasa T, Nakao K. Vascular endothelial growth factor suppresses C-type natriuretic peptide secretion. *Hypertens (Dallas, Tex 1979)*. 1996;27(3 pt 2):811–815. doi:10.1161/01.hyp.27.3.811.
22. Ohta S, Takeuchi M, Deguchi M, Tsuji T, Gahara Y, Nagata K. A novel transcriptional factor with Ser/Thr kinase activity involved in the transforming growth factor (TGF)-beta signalling pathway. *Biochem J*. 2000;350(pt 2):395–404.