**A Janus-faced role for atrial natriuretic peptide in myocardial infarction?**

Adrian J. Hobbs

*Professor of Cardiovascular Pharmacology, William Harvey Research Institute, Barts & The London School of Medicine, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK. Email:* [*a.j.hobbs@qmul.ac.uk*](mailto:a.j.hobbs@qmul.ac.uk)*; Tel: +44 (0)207 882 5778*

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The biological significance of atrial natriuretic peptide (ANP) has been studied exhaustively from a physiological, pathological, pharmacological and diagnostic standpoint. This member of the natriuretic peptide family exerts a profile of complementary effects in the cardiovascular system, including natriuretic, diuretic, vasodilator, anti-RAAS, anti-hypertrophic, and lipolytic activity to regulate blood volume, blood pressure, and maintain cardiac structure & function1. Despite this extensive cytoprotective profile pharmacological manipulation of ANP, and natriuretic peptides in a more general sense, has not been exploited therapeutically to the extent that might have been anticipated. Recombinant ANP (Carpertide) and brain natriuretic peptide (BNP; Nesiritide) are licensed for treatment of acute heart failure in some territories without overwhelming evidence from large-scale, randomised controlled trials. Only very recently, LCZ696, a novel molecule combining the angiotensin receptor blocker valsartan with sacubitril, an inhibitor of neutral endopeptidase (NEP; an enzyme that hydrolyses and inactivates natriuretic peptides and a number of other vasoactive mediators), was shown to produce an approximate 20% reduction in cardiovascular death and hospitalisation compared to enalapril in patients with heart failure, and is now licensed for this disorder2 (LCZ696 produced an increase in urinary cyclic guanosine-3’-5’-monophosphate (cGMP) levels inferring that it was increased natriuretic peptide biology that underpinned its therapeutic effect). Besides these limited examples, ANP and BNP have also been shown to reduce infarct size and/or improve left ventricular function following acute myocardial infarction (MI) in early phase trials3. Could this represent a further indication in which harnessing natriuretic peptide bioactivity will be valuable therapeutically? In an intriguing and pertinent study published in this issue of *Circulation Research,* Chen et al.4present important new data that add to the understanding of the role(s) of ANP (and BNP) following MI and urge caution that although the net effect might be positive it is not necessarily all one way traffic.

Using a unique transgenic mouse line with endothelial-restricted deletion of guanylyl cyclase (GC)-A, the cognate receptor for ANP and BNP, the authors demonstrate that ANP triggers a GC-A/cGMP axis following permanent coronary artery ligation that increases endothelial permeability and facilitates neutrophil extravasation into the infarcted myocardium, worsening severity; this is paralleled by an increase in CXCL1/KC mRNA, a key neutrophil attractant chemokine, and higher expression of intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and both E- and P- selectin. Endothelial GC-A deletion elicits favourable effects on each of these processes, but this advantageous profile is surprisingly not recapitulated in animals lacking endothelial cGMP-dependent protein kinase (cGK) I; such observations imply that an alternate cGMP ‘effector’ is pivotal in mediating the GC-A-driven exacerbated pathology, and Chen et al.4 provide convincing evidence to establish this downstream target as cGMP-stimulated phosphodiesterase (PDE) 2. This member of the PDE superfamily hydrolyses both cGMP and cAMP, and Chen et al.4 report that PDE2 expression is up-regulated in endothelial cells by hypoxia and TNFα (with simultaneous down-regulation of cGKI) and results in attenuated cGMP formation in response to ANP, an effect restored by addition of the selective PDE2 inhibitor (PDE2i) BAY 60-7550. Crosstalk with the cAMP system is also demonstrated using a novel FRET sensor. Using this approach, ANP is shown to produce a rise in cellular (specifically sub-membrane) cAMP that is blocked in the presence of TNFα via up-regulation of PDE2; again, BAY 60-7550 is able to reverse this phenotype. To substantiate these findings, intravital microscopic analysis of dextran leak from the cremaster circulation revealed that the increase in permeability in response to a threshold dose of TNFα is significantly enhanced by ANP (and BNP), indicating cGMP/cAMP crosstalk, an effect lost in endothelial GC-A KO or mice treated with BAY 60-7550; extravasation of leukocytes followed an essentially identical pattern. Collectively, these observations demonstrate that release of ANP following MI can facilitate a GC-A/cGMP/PDE 2/cAMP signalling cascade that promotes endothelial permeability, facilitates neutrophil recruitment, and aggravates injury.

These new findings add further complexity to the pathological roles of ANP in the cardiovascular system, and particularly those related to MI. GC-A activation has traditionally been associated with an endothelial barrier protective function5; certainly, from a pharmacological perspective, molecules that potentiate the biological activity of natriuretic peptides, such as NEP inhibitors, promote endothelial barrier integrity and prevent leukocyte recruitment to extravascular sites of injury and inflammation6. In the present study4, the hyperpermeability induced by ANP is ultimately elicited via breakdown of cAMP (which is primarily thought to be protective in terms of endothelial permeability and barrier function7). Crosstalk between the cGMP and cAMP systems is determined ostensibly by the activity of two cGMP-regulated cAMP-hydrolysing PDEs; PDE2 contains a GAF-B domain at its N terminus which binds cGMP to allosterically up-regulate activity, whilst for PDE3 cGMP is a high affinity inhibitor at the substrate binding site due to its low hydrolytic Vmax (versus cAMP)8. Previous studies have intimated that inhibition of PDE3 and reduced permeability tends to occur at lower cGMP concentrations whereas ANP-facilitated cAMP hydrolysis and increases in permeability predominate at higher cGMP concentrations7. This has important implications for MI since hypoxia and TNFα both promote PDE2 expression and the pathological release of ANP and/or BNP in this setting must be commensurate with sufficient GC-A stimulation to promote activation of PDE2. However, endothelial-specific deletion of GC-A *per se* increases intravascular volume5, suggesting that even under physiological conditions ANP/GC-A/cGMP primarily couples with PDE2 rather than PDE3, at least in the endothelium. Yet, the picture is more complicated; in the lung pharmacological administration of ANP protects against oedema and ANP KO mice exhibit augmented LPS-induced injury9. In addition, various agents that promote endothelial permeability appear to differentially affect expression of PDE2 versus PDE3. For example, in the study of Chen et al.4 TNFα increased PDE2 expression thereby favouring enhanced cAMP (and cGMP) breakdown in the face of GC-A activation; TNFα also concomitantly reduces cGKI and PDE3 expression to tip the balance even further. However, other mediators that increase endothelial permeability, such as thrombin, can activate PDE310; in this scenario, the net result on permeability and leukocyte flux might be quite different. These disparate outcomes almost certainly illustrate a shift between interaction of cGMP with PDE2 or PDE3 in organ-specific environments.

The spatial and temporal differences in cyclic nucleotide dynamics demonstrate the need to more fully understand the phenomenon of compartmentalised signalling before it will be possible to elucidate why natriuretic peptides have the capacity to exert both positive and negative effects on endothelial permeability and barrier function. Regardless, the interchange between PDE2 and PDE3 appears to proffer a flexible, but fine-tuned regulation of many functional consequences of cyclic nucleotide signalling and is now a relatively well-established phenomenon, particularly in the myocardium. For instance, in human atrial myocytes lower concentrations of cGMP augment L-type Ca2+ channel activity through inhibition PDE3, whereas higher concentrations of cGMP reduce such currents via PDE2 activation11. Furthermore, the principal PDE responsible for cGMP/cAMP breakdown can shift as a result of disease. This facet is highlighted by the observation that in healthy myocardium cGMP generated by natriuretic peptides is almost exclusively regulated by PDE212, whereas in the failing heart, C-type natriuretic peptide (CNP) at least, can potentiate β-adrenoceptor-induced increases in force of contraction via cGMP-driven inhibition of PDE313.

Do the findings of Chen et al.4 imply that PDE2 inhibitors might be a useful adjunctive therapy for MI to optimise the beneficial effects of cardiac natriuretic peptide bioactivity? Perhaps, but the outcome is difficult to predict. In the pulmonary vasculature and right ventricle PDE2 expression is downregulated in response to hypoxia, and enzyme inhibitors are effective in preventing and reversing the pulmonary hypertensive phenotype, lowering pulmonary pressure, limiting pulmonary vascular remodelling and abrogating the RV hypertrophy14; in a similar context, blockade of PDE2 reduces the development of pulmonary oedema following acute lung injury by accentuating an cAMP-driven reduction in permeability15. In sharp contrast, in the failing left ventricle, upregulation of PDE2 has been suggested to represent a protective mechanism limiting detrimental cAMP-dependent β-adrenergic signalling16. In actual fact, this right-left heart discrepancy might be explained, at least in part, by differential activity of cAMP and crosstalk with cGMP systems since long term potentiation of cAMP-dependent pathways (e.g. β-agonists, PDE3i) increases mortality in patients with LV dysfunction, whereas pharmacologically targeting cAMP signalling via the use of prostacyclin analogues offers a survival advantage in right heart failure (i.e. PH patients). Thus, the organ-, tissue- and cell- specific nature of the pathways involved suggests that if the injurious effect of GC-A/cGMP activation to increase endothelial permeability and leukocyte extravasation following MI is to be abrogated therapeutically, then specific targeting of endothelial PDE2 might be the ultimate, albeit challenging, goal. Furthermore, since the study of Chen et al.4 explored barrier function and leukocyte flux following MI primarily from the perspective of endothelial GC-A deletion, it would also be interesting to know if by employing pharmacological GC-A stimulators (i.e. agonists) in this context it might be possible to optimise dose and time of administration to exploit the recognized salutary effects of ANP and BNP (e.g. vasodilator, natriuretic/diuretic, sympatholytic, anti-RAAS, anti-hypertrophic & anti-fibrotic) whilst minimising or avoiding activation of PDE2?

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