Visualizing the Basis for Paracrine Natriuretic Peptide Signaling in Human Heart

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Since the discovery of atrial natriuretic factor 25 years ago,1 there has been an explosion of research elucidating the basic biology of endogenous natriuretic peptides. Among other things, this work has established atrial natriuretic factor as one of a family of structurally similar endogenous natriuretic peptides with complex and distinct functional roles in maintaining normal homeostasis and responding to pathological circumstances.

The first identified member of this family, atrial natriuretic peptide (ANP), is synthesized and secreted in the cardiac atria under normal conditions and by the ventricular myocardium during fetal development, hypertrophy, or heart failure. BNP, a distinct natriuretic peptide first isolated from the porcine brain, is preferentially synthesized and secreted by ventricular cardiac myocytes and, like ANP, exhibits increased expression during hypertrophy and heart failure. In contrast, C-type natriuretic peptide (CNP) is mainly produced by vascular endothelial cells and neurons, whereas urodilatin is synthesized and secreted by renal cells. The last identified member of this peptide family is dendraosipis natriuretic peptide (DNP), which was first isolated from the venom of the Green Mamba snake.2 Although immunoreactivity to DNP has been identified in several human tissues3 and circulating plasma,4 the gene encoding this natriuretic peptide has not yet been identified within the human genome.

Actions of natriuretic peptides are mediated through binding to 3 distinct natriuretic peptide receptors (NPRs) that are located on the cell surface and bind endogenous ligands with varying specificities and affinities (Figure).5 Two of the receptors, NPR-A and NPR-B, have an extracellular (ligand-binding) domain linked to an intracellular (catalytic) domain with guanylate cyclase activity. Consequently, binding to NPR-A and NPR-B triggers increases in guanylate cyclase activity, increased intracellular cGMP, and downstream signaling and responses. The third natriuretic peptide receptor, NPR-C, has no catalytic domain or guanylate cyclase activity, and contributes to the clearance of natriuretic peptides from the circulation. NPR-A receptors exhibit high affinity for ANP, BNP, and DNP, but have relatively low affinity for CNP. In contrast, NPR-B receptors bind with high affinity to CNP, but not ANP or BNP. The NPR-C receptor binds ANP, BNP, and CNP with approximately equal affinity. Differences in the tissue distributions of NPR subtypes and the potential for disease-related alterations further complicate the biology of endogenous natriuretic peptides.5

Early work examining the functional biology of ANP and BNP focused on their roles as endocrine substances. In these constructs, increases in natriuretic peptide synthesis and secretion in response to acute or chronic cardiac chamber distension resulted in increased circulating levels of the secreted peptides and effects at remote target organs such as the kidney, adrenal, or vasculature. Though paracrine effects were implicated when CNP and NPR-B receptors were both found within the vasculature,6 there was initially little support for functionally significant local cardiac effects of cardiac derived ANP or BNP. Indeed, during infusions of exogenous natriuretic peptides, decreases in cardiac output and preload were attributed to natriuresis, venodilation, and decreases in systemic vascular resistance. An absence of data indicating expression of NPR-A receptors in the heart supported the impression that endogenous natriuretic peptides had no direct effects on the myocardium.

However, the later advent of pharmacologic and genetic NPR-A inhibition implicated paracrine and autocrine actions of cardiac natriuretic peptides, even when data supporting myocardial NPR-A were lacking.7 Studies using a specific NPR-A receptor antagonist (HS-142) suggested direct cardiac actions of natriuretic peptides. In cultured myocytes, HS-142 modulated secretion of ANP8 and increased cellular hypertrophy, particularly in the setting of α1-adrenergic stimulation via phenylephrine.9 In vivo, HS-142 blocked coronary vasodilation responses to exogenous ANP10 and induced immediate slowing of ventricular relaxation and decreases in coronary blood flow suggesting direct lusitropic and vasodilating actions of NPR-A binding by endogenous natriuretic peptides.11 Complementing pharmacological approaches, transgenic mice lacking NPR-A receptor exhibit increases in cardiac mass out of proportion to their degree of hypertension,12 and cultured myocytes from NPR-A−/− mice are hypertrophied.13 Together, these studies suggested functionally important autocrine and paracrine cardiac actions of endogenous natriuretic peptides despite the lack of direct evidence for expression and cellular localization for NPR-A receptors in the heart.

In this context, the article by Singh et al in the current issue of Circulation Research examines the distribution and binding kinetics of the natriuretic peptide type A (NPR-A) receptor in human heart tissue.14 The authors developed a novel radiolabeled DNP analogue,125I-DNP, and used this to
localized high affinity ANP-binding sites in the human heart. They complemented these studies with immunocytochemical studies with a commercially available anti–NPR-A antibody. For the first time, these studies demonstrated that NPR-A receptors are expressed on cardiomyocytes and coronary vascular smooth muscle cells, in addition to the endocardial expression previously reported.15,16 These studies also indicated that for 125I-DNP binding density was reduced in hearts from patients with ischemic heart disease, but not in those derived from patients with nonischemic heart failure.

In these new studies, multiple approaches were used to demonstrate that 125I-DNP is specifically binding to NPR-A receptors. 125I-DNP staining was displaced by natriuretic peptides but not by other vasoactive peptides, consistent with 125I-DNP binding to specific NPRs. The NPR binding affinity was greatest for DNP, less for BNP and ANP, and lowest for CNP, a pattern consistent with NPR-A, rather than NPR-B or NPR-C, binding. Moreover, 125I-DNP binding did not colocalize with the vascular endothelium, another finding consistent with NPR-A rather than NPR-B binding. Finally, the conclusion that 125I-DNP is a specific NPR-A ligand is concordant with the reported actions of exogenous DNP in inducing natriuresis and vasodilation, responses typically associated with NPR-A activation.3,17 The high affinity of NPR-A for DNP over the other natriuretic peptides also suggests a therapeutic potential for this more recently characterized natriuretic peptide.

The myocardial localization of the NPR-A receptors by Singh et al confirms and substantially extends previous studies. Early studies using 125I-ANP identified all NPR subtypes in endocardium of the right and left ventricles, but these studies failed to identify myocardial NPR-A expression.15,16 A later study reported visualization of 125I-ANP binding in explanted bovine hearts, but neither distinguished the receptor subtypes nor confirmed endocardial 125I-ANP binding.18 In contrast, using 125I-DNP autoradiography, Singh et al observed NPR-A binding throughout the myocardium of both ventricles and both atria along with the endocardium and smooth muscle of the intramyocardial blood vessels. In histologically normal coronary arteries, 125I-DNP binding was evident in the vascular smooth muscle but not the endothelium. RT-PCR and immunohistochemical techniques confirmed the NPR-A expression in cardiac myocytes and vascular smooth muscle cells as suggested by the 125I-DNP autoradiography. The authors speculate that resistance of 125I-DNP to degradation by neutral endopeptidase19 and its preferential binding to NPR-A explains the success of this probe in visualizing NPR-A receptors though 125I-ANP failed to do so.

Still more intriguing is the reduced 125I-DNP binding density in hearts obtained from individuals with ischemic heart disease. Earlier reports of changes in mRNA abundance for NPRs in rat models of volume overload hypertrophy20 and experimental myocardial infarction21 did not assess receptor binding dynamics. Other studies reported decreased 125I-ANP binding and agonist-induced cGMP generation in the right ventricular endocardium of rats with experimental pulmonary hypertension, but did not detect NPRs within the myocardium.16 In contrast, Singh et al demonstrate disease-related changes in both the left ventricles and coronary arteries of hearts from patients with advanced ischemic heart disease. The decreased NPR-A binding density in smooth muscle from atherosclerotic arteries suggests the likelihood of reduced coronary, and perhaps extracoronary, vasodilator responses to endogenous and exogenous ANP and BNP. Similarly, downregulation of NPR-A density in the myocardium of patients with severe ischemic heart disease could result in reduced myocardial actions of ANP and BNP. Based on studies with NPR-A blockade and NPR-A−/− mice, the NPR-A downregulation observed in patients with ischemic heart disease might be associated with a propensity toward myocyte hypertrophy and fibrosis,9,22 particularly in the setting of unopposed renin–angiotensin system activation.13 At the same time, the absence of reduced 125I-DNP binding density in coronary arteries and myocardium from patients with idiopathic dilated cardiomyopathy indicates that the changes observed in ischemic heart disease are not solely a consequence of cardiomyopathy or heart failure per se. Regulation of the other two natriuretic peptide subtypes, NPR-B and NPR-C, in the failing heart is an important remaining question with both biological and therapeutic relevance.

Beyond the specific findings reported in this manuscript, the use of 125I-DNP binding as a reagent for identifying and characterizing NPR-density provides opportunities for future research. For example, future studies may examine the distribution and regulation of NPR-A within the kidney and peripheral vasculature by using 125I-DNP binding studies. Such explorations will be particularly informative in settings with altered physiological responses to NPR-A ligands, such as the reduced renal responsiveness observed in advanced heart failure.

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References


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