

Natriuretic Peptides in the Regulation of Cardiovascular Physiology and Metabolic Events

Risto Kerkelä, MD, PhD; Johanna Ulvila, PhD; Johanna Magga, PhD

Atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) are the known members of the mammalian natriuretic peptide system. The discovery of natriuretic peptides (NPs) dates back to 1981, when de Bold et al. found that administration of atrial extracts into intact rats causes diuresis and natriuresis.¹ In 1983–1984, ANP was then isolated and purified, and the amino acid sequence was determined in rats and humans. Whereas atrial ANP levels are higher than those in ventricles, the ventricular myocardium becomes a major source for circulating ANP in failing hearts owing to larger mass.² In 1988, a homologous peptide with similar biological activities was isolated from porcine brain and named BNP.³ BNP is expressed in both adult atria and ventricles, but is mainly released from the ventricles.

The key stimulant for release of ANP and BNP from the heart is myocardial stretch, whereas the third family member, CNP, is mainly released from endothelial cells in response to various cytokines² as depicted in Figure 1. Classically, ANP and BNP exert diuretic, natriuretic, and hypotensive actions, and genetic variations of the *NPPA-NPPB* locus that increase the circulating levels of ANP and BNP in patients have been shown to offer protection from hypertension.⁴ In the clinical setting, analysis of serum levels of ANP and BNP is used for diagnosis of heart failure (HF) and it has also been suggested that analysis for circulating levels of ANP and BNP may be useful in monitoring the efficacy of therapies in treatment of patients with HF. Notably, though, treatment of patients with β -blockers, which reduces cardiovascular morbidity and mortality especially in post-MI (myocardial infarction)

patients, is associated with elevated circulating levels of ANP and BNP.⁵ Recombinant human ANP and BNP have been approved for treatment of acutely decompensated congestive HF in Japan and the United States, respectively. In addition to renal effects, during the last decade it has become evident that NPs have direct effects in multiple other tissues and are involved in regulation of a variety of biological processes, such as cardiac hypertrophy, fibrosis, metabolism, angiogenesis, and cardiomyocyte viability. In this review, we summarize the recent findings of the cardiovascular effects of NPs with emphasis on effects of NP signaling on cardiac structure and function.

Natriuretic Peptides and Their Receptors

All NPs are initially synthesized as preprohormones and their subsequential cleavage results in formation of biologically active 28-amino-acid ANP, 32-amino-acid BNP, and 22- and 53-amino-acid CNP.⁶ ANP is secreted from cardiomyocytes as active hormone, whereas BNP is secreted from cardiomyocytes as a 108-amino-acid prohormone (proBNP). In circulation, glycosylated proBNP is gradually deglycosylated and further processed by the proNP convertases, corin or furin, to inactive NT-proBNP1-76 and active BNP1-32.⁷ Evolutionary studies suggest that CNP, which, differently from ANP and BNP, does not stimulate natriuresis, is the most ancient family member.

The NPs exert their actions through interaction with their cell surface receptors. To date, 3 NP receptors (NPRs) have been identified, and they can be divided into 2 major classes: guanylyl cyclase-coupled receptors A and B (GC-A and GC-B, or NPRA and NPRB) and a natriuretic peptide clearance receptor (NPRC).⁶ NPRA and NPRB both exist as homodimers and the intracellular domain consists of a kinase homology domain, a dimerization domain, and a C-terminal catalytic domain. Activation of NPRA and NPRB induce elevation of intracellular cyclic guanosine monophosphate (cGMP) and activation of its key effector molecule, protein kinase G (PKG), which is largely thought to exert the cardioprotective effects of NPs.⁸ NPRC, on the other hand, lacks the intracellular guanylate cyclase domain and has been shown to signal to

From the Department of Pharmacology and Toxicology, Research Unit of Biomedicine, University of Oulu, Finland (R.K., J.U., J.M.); Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Finland (R.K.).

Correspondence to: Risto Kerkelä, MD, PhD, Department of Pharmacology and Toxicology, Research Unit of Biomedicine, University of Oulu, P.O. Box 5000, FIN-90014 Oulu, Finland. E-mail: risto.kerkela@oulu.fi
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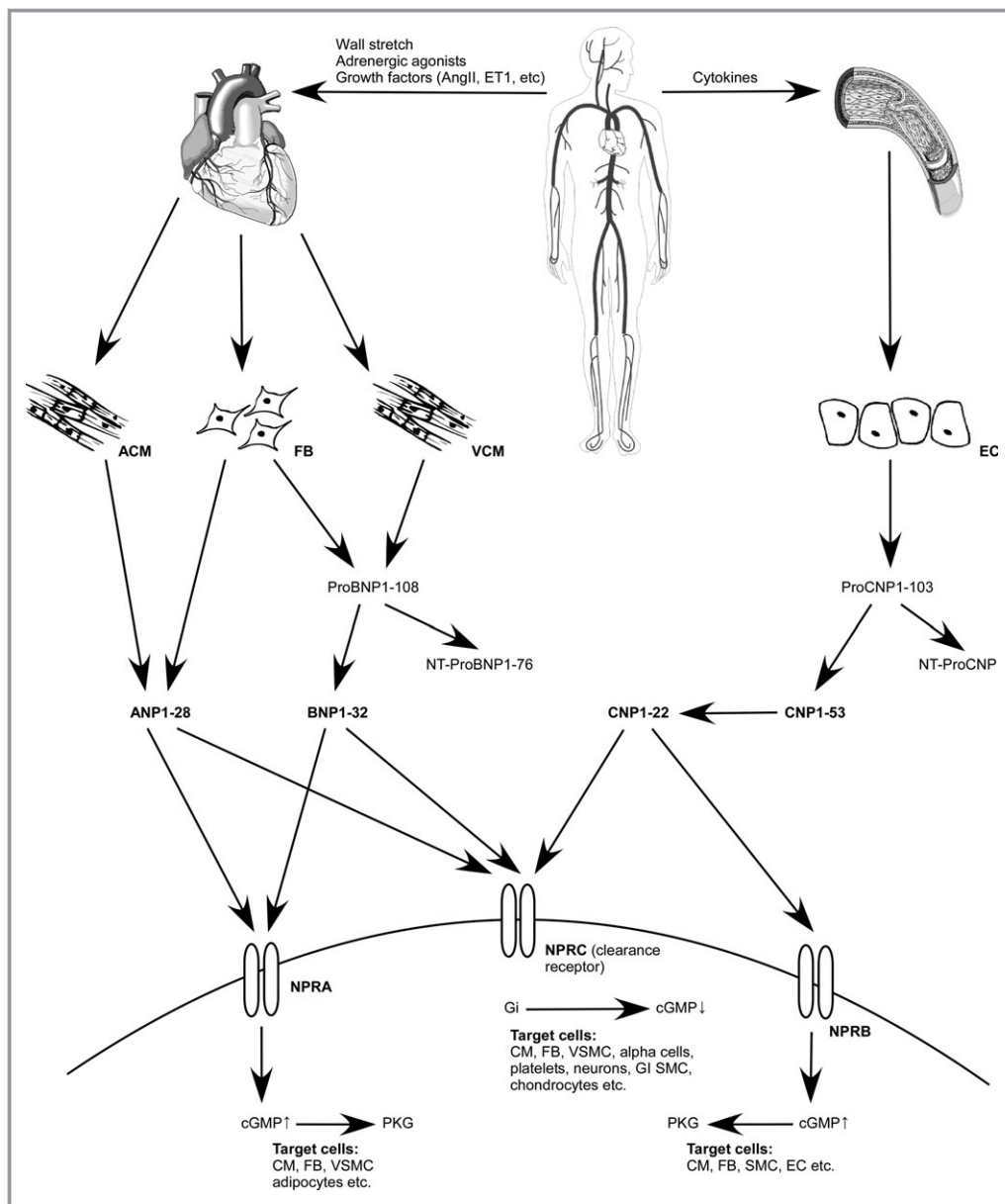


Figure 1. NPs are secreted from different cell types or tissue sites and signal through guanylyl cyclase or G-protein-coupled receptor in various target cells throughout the body. ACM indicates atrial cardiomyocytes; Ang II, angiotensin II; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; cGMP, cyclic guanosine monophosphate; CM, cardiomyocyte; CNP, C-type natriuretic peptide; EC, endothelial cell; ET-1, endothelin-1; FB, fibroblast; GI, gastrointestinal; Gi, inhibitory G protein; NPR, natriuretic peptide receptor; NT-Pro, N-terminal propeptide; PKG, protein kinase G; SMC, smooth muscle cell; VCM, ventricular cardiomyocytes; VSMC, vascular smooth muscle cell.

inhibitory guanine nucleotide-binding protein (inhibitory G protein).⁹ Soluble guanylyl cyclase, on the other hand, is activated by nitric oxide and was recently shown to mediate the protective effects of β 3-adrenergic receptor activation on left ventricle (LV) remodeling.^{10,11} NP activation, secretion, and signaling in different cell types are summarized in Figure 1.

Juxtamembrane portions of NPRA and NPRB contain multiple phosphorylation sites, and phosphorylation of those

sites has been shown to regulate receptor sensitivity. ANP and BNP primarily bind and signal through NPRA, whereas CNP binds to NPRB, and the clearance receptor, NPRC, binds all NPs.^{6,12} In the myocardium, all three NP receptors have been shown to be expressed in both cardiomyocytes and cardiac fibroblasts.¹³ NPRA stimulation by ANP or BNP has been shown to induce an increase in cGMP levels in cardiomyocytes, whereas NPRB stimulation by CNP was without effect.¹³ In cardiac fibroblasts, both ANP and CNP

stimulation induced an elevation in cGMP levels, thus suggesting that both NPRA and NPRB are functional.¹³ This indicates some discrepancy in NP signaling between cardiomyocytes and fibroblasts in the heart.

Numerous studies have demonstrated the central role for NPs in regulation of blood pressure. Genetic studies in humans have suggested a role for NPs regulating cardiac hypertrophy and fibrosis, thus increasing interest in investigating NPs as targets for HF therapy. A study conducted in Italy showed that carriers of allele 664C>G in ANP promoter, which results in lower levels of circulating ANP, had increased LV mass index and increased LV wall thickness.¹⁴ In another study, deletion of 5'-flanking region of the human NPRA gene found in Japanese patients, which reduced the transcriptional activity of the gene by 30%, appeared to increase susceptibility to hypertension and LV hypertrophy.¹⁵ A study conducted on a cohort of a nondiabetic Swedish population showed that subjects with the minor allele of rs5068 at the *NPPA-NPPB* locus, which results in higher circulating levels of ANP and BNP,⁴ was associated with reduced incidence of LV

hypertrophy.¹⁶ The same genetic ANP variant also showed protection against metabolic syndrome. These findings indicate that NPs may exert multiple cardioprotective effects, mediated by NP receptors expressed in myocardium, vasculature, and in a variety of other tissues, as indicated in Figure 2.

Regulation of ANP and BNP Gene Expression

In addition to mechanical stretch, multiple other stimuli, such as angiotensin II (Ang II), endothelin-1 (ET-1), as well as adrenergic agonists isoproterenol and phenylephrine induce ANP and BNP transcription and secretion from cardiomyocytes. Mitogen-activated protein kinases (MAPKs) and, especially, extracellular signal-regulated kinase (ERK), which is activated by virtually all hypertrophic stimuli in cardiomyocytes, represent the central pathway regulating transcription of ANP and BNP.¹⁷ Constitutive activation of MEK1 (upstream activator of ERK) in myocytes induces cardiac hypertrophy and NP expression,¹⁸ but loss of ERK from cardiomyocytes is

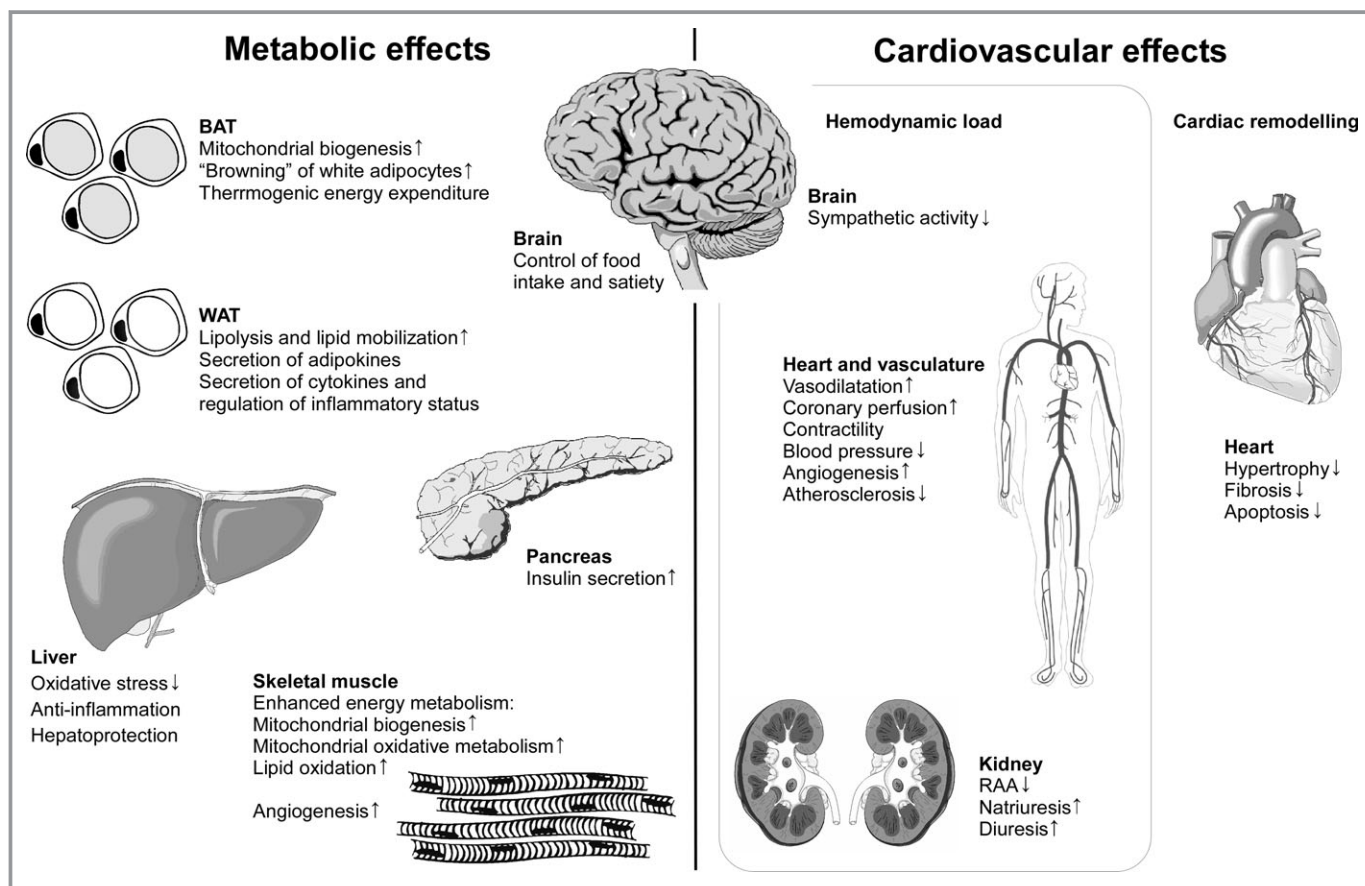


Figure 2. NPs regulate key functions of the cardiovascular system. NPs also exert various functions on metabolic events, including enhanced energy metabolism, favorable body fat profile, and increased insulin sensitivity, the factors closely associated with development of cardiovascular diseases. BAT indicates brown adipose tissue; NP, natriuretic peptide; RAA, renin-angiotensin-aldosterone system; WAT, white adipose tissue.

not sufficient to block stress-induced hypertrophic response.¹⁹ In addition to ERK, p38 MAPK has been shown to regulate NP expression in primary cardiomyocyte culture. Constitutive activation of p38 β induces both ANP and BNP gene expression, and inhibition of p38 β by an adenovirus-overexpressing dominant-negative p38 β blocks ET-1-induced BNP transcriptional activity.²⁰

GATA4 appears to be the key transcriptional effector regulating transcription of ANP and BNP. GATA4 is phosphorylated at Ser105 by both ERK and p38,^{21–23} regulating its DNA-binding activity and transcriptional activity.^{22–26} In addition to GATA4, a number of other transcriptional regulators, such as nuclear factor of activated T-cells (NFAT), NKX-2.5, Elk-1, activator protein 1, myocardin, serum response factor, and nuclear factor kappa B, have been shown to participate in transcriptional regulation of BNP.^{20,27–32} Several studies have also indicated a central role for M-CAT element, a thyroid-responsive element and shear stress-responsive element on the BNP promoter regulating BNP transcriptional activity.^{33–36} In conclusion, the factors regulating cardiac hypertrophy, mainly MAPKs and GATA4 transcription factor activity, are also associated with regulation of NP expression levels.

ANP and BNP in the Failing Heart

Synthesis and secretion of ANP and BNP in the heart are increased during hemodynamic overload and cardiac remodeling both in humans and in experimental animal models. Cardiac remodeling is a hallmark in the progression of many cardiovascular diseases and is characterized by LV hypertrophy and cardiac fibrosis. Accumulation of cardiac interstitial fibrosis leads to stiffening of the LV wall, worsening of LV systolic and diastolic function, and predisposes to cardiac arrhythmias. Cardiac fibroblasts not only produce extracellular matrix proteins, but also secrete a variety of growth factors that mediate an interplay between cardiac fibroblasts and cardiomyocytes. Several growth factors, such as Ang II, ET-1, and platelet-derived growth factor, and various cytokines, such as transforming growth factor-beta (TGF β) and connective tissue growth factor (CTGF), have been proposed to act in either para- or autocrine fashion between fibroblasts and cardiomyocytes to stimulate cardiac remodeling.^{37,38} Multiple studies now indicate that NPs also directly affect the function of cardiac fibroblasts and cardiomyocytes. Cardiovascular effects of NPs are summarized in Figure 2.

First evidence for the role of NPs regulating fibroblast function originate from in vitro studies. Furthermore, transgenic mouse models have been invaluable tools in investigating the roles of NPs in cardiac biology. Especially, the experimental design utilizing mice with ANP, BNP, or NPRA

deficiency have proved fruitful. Cardiac remodeling has been investigated in mice lacking the *Npr1* gene (encoding for NPRA), which effectively blunts the effects of ANP and BNP. In vitro and in vivo studies concerning cardiovascular effects of natriuretic peptides are represented in Table 1. These data categorically demonstrate that NPRA signaling is an intrinsic signaling mechanism in the myocardium that inhibits both cardiac hypertrophy and fibrosis. Notably, ANP and BNP appear to inhibit cardiac fibrosis by modulating renin-angiotensin-aldosterone (RAA) system signaling.

Apart from reducing hypertrophy and fibrosis, NPRA activation may also benefit cardiac function in HF, as demonstrated in animal models of HF (Table 1). In humans, treatment of HF patients with recombinant BNP was shown to cause a dose-related decrease in pulmonary-capillary wedge pressure.³⁹

The NP/NPRA system also acts as regulator of angiogenesis in the heart and skeletal muscle. Thus, in addition to reducing blood pressure, cardiac hypertrophy, and fibrosis, NPs may also signal to enhance angiogenesis and help neovascularization in injured muscle. It remains to be investigated whether enhanced NPRA signaling induces neovascularization and injury repair in the infarcted myocardium.

Molecular Mechanisms of Antihypertrophic and Antifibrotic Effects of ANP and BNP

Deletion of Ang II type 1a receptor gene (*Agtr1*) largely reverses the enhanced myocardial fibrosis observed in mice with *Npr1* deficiency. In addition to Ang II, TGF β 1 also appears to play a role in antifibrotic effect of NPRA activation. ANP/BNP signaling by NPRA, cGMP, and PKG attenuate TGF β 1, apparently in an ERK-dependent manner, and inhibit fibrotic response induced by TGF β in cardiac fibroblasts. Specific molecular mechanisms of antihypertrophic and -fibrotic effects of ANP and BNP are represented in Table 2. Another mechanism suggested for antifibrotic effect of NPs is reduced cardiac RAA system. ANP is also linked to regulation of GATA4 activity, and a number of other signaling pathways in cardiomyocytes have been shown to mediate the antihypertrophic effects of NPs (Table 2).

Collectively, ANP and BNP regulate a number of key elements associated with cardiac fibrosis, such as TGF β 1 and ET-1, as well as central hypertrophy-related regulators, such as transcription factor GATA4 and local cardiac RAA system. Interestingly, the actions of NPs also reach to regulation of cellular ions, mainly calcium, by numerous divergent mechanisms. In addition, NPs also regulate the activity of G-protein-coupled receptors by activation of guanylyl cyclase receptor and formation of cGMP.

Table 1. Selected Cardiovascular Effects of ANP and BNP in In Vitro and In Vivo Animal Models

Model	Biological Effect	References
Regulation of blood pressure		
<i>Nppa</i> gene deletion	Genetically reduced production of ANP leads to salt-sensitive hypertension.	40
Oral administration of conjugated BNP in dogs	BNP reduces blood pressure and increases natriuresis in normal dogs and in acute Ang II-induced hypertension.	41
Regulation of cardiac fibrosis, cardiac hypertrophy, and cardiac remodeling		
NRCF	ANP and BNP inhibit Ang II-induced proliferation of fibroblasts by inhibiting ET-1 gene expression.	42
NRCF	ANP, BNP, and CNP inhibit vasoactive peptide or growth-factor-induced proliferation of fibroblasts.	43
NRCM and NRCF	ANP inhibits NE-induced protein synthesis in cardiomyocytes and DNA synthesis in fibroblasts by cGMP-mediated inhibition of calcium channels.	44
<i>Nppb</i> gene deletion	Genetically reduced production of BNP leads to development of multifocal fibrotic lesions in subendocardial regions of ventricles in young adults and exaggerates fibrosis in response to hemodynamic overload induced by TAC.	45,46
<i>Nppa</i> gene deletion × cardiac overexpression of dnCREB	Genetically reduced production of ANP increases adverse LV remodeling and cardiac fibrosis, and dose-dependently decreases survival in a mouse model of dilated cardiomyopathy.	47
<i>Npr1</i> gene deletion	NPRA deficiency leads to modest increase in blood pressure, but results in severe cardiac hypertrophy, fibrosis, and LV dysfunction. Normalizing the blood pressure with antihypertensive therapy does not alleviate the adverse effects on cardiac remodeling, indicating some direct hypertrophic mechanism mediated by deficient NPRA signaling.	48–51
Cardiac <i>Npr1</i> gene deletion, or cardiac overexpression of NPRA	Cardiac deletion of <i>Npr1</i> increases cardiomyocyte size and, conversely, cardiac overexpression of NPRA decreases cardiomyocyte cross-sectional area. Transgenic mice with cardiomyocyte-specific deletion of <i>Npr1</i> develop more-severe cardiac fibrosis, hypertrophy, and LV dysfunction in response to TAC-induced pressure overload.	49,52
<i>Npr1</i> gene deletion × <i>Agtr1</i> gene deletion	NPRA deficiency combined with deficient Ang II type 1a receptor gene blocks MI-induced development of cardiac fibrosis, but not development of hypertrophy.	53
Cardiac overexpression of dnNPRA	Disruption of functional NPRA results in enhanced cardiac hypertrophy and fibrosis in response to chronic hypertension induced by suprarenal aortic banding.	54
Regulation of cardiac function in heart failure		
Subcutaneous administration of BNP in dogs	Augmenting NPRA signaling by administration of BNP improves cardiac output while reducing systemic vascular resistance and cardiac filling pressure in pacing-induced chronic HF.	55
Intravenous administration of cardiotropic AAV9 carrying proBNP in rats	Long-term cardiac proBNP delivery improves both systolic and diastolic function and reduces LV mass in spontaneously hypertensive rats.	56
Intracardiac administration of adenovirus carrying BNP in rats	BNP gene delivery to LV reduces cardiac fibrosis, increases capillary density, and improves LV function in rats after experimental MI.	57
Regulation of angiogenesis		
<i>Npr1</i> gene deletion	Vascular regeneration in a hindlimb ischemia model is impaired in mice deficient for NPRA. NPRA deficiency does not affect the mobilization of vascular progenitor cells from bone marrow.	58,59
<i>Npr1</i> gene deletion, endothelial or smooth muscle <i>Npr1</i> gene deletion	Vascular regeneration in a hindlimb ischemia model is impaired in mice with systemic or endothelial-cell-specific knockout of NPRA. Endothelial-cell-specific deletion of <i>Npr1</i> shows diminished angiogenesis in the heart, mild fibrosis, and diastolic dysfunction in response to TAC. NPRA deficiency in smooth muscle cells does not affect ischemic neovascularization.	59
Overexpression of BNP	Mice overexpressing BNP shows enhanced neovascularization in response to hindlimb ischemia.	60

AAV indicates adeno-associated virus serotype 9; *Agtr1*, Ang II, angiotensin receptor II type 1; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; cGMP, cyclic guanosine monophosphate; CNP, C-type natriuretic peptide; CREB, cyclic adenosine monophosphate response element-binding protein; dn, dominant negative; ET-1, endothelin 1; HF, heart failure; LV, left ventricle; MI, myocardial infarction; NE, norepinephrine; *Nppa*, atrial natriuretic peptide encoding gene; *Nppb*, B-type natriuretic peptide encoding gene; NPRA, natriuretic peptide receptor A; NRCF, neonatal rat cardiac fibroblasts; NRCM, neonatal rat cardiomyocytes; TAC, transverse aortic constriction.

CNP in the Diseased Heart

In the cardiovascular system, NPRB is expressed mainly in endothelial cells and smooth muscle cells and is primarily

activated by CNP (Figure 1). Membrane preparations from mouse hearts subjected to pressure overload show slightly higher guanylyl cyclase activity in response to saturating concentrations of ANP than upon stimulation by CNP.⁷⁶

Table 2. Selected Molecular Mechanisms of Antihypertrophic and Antifibrotic Effects of ANP and BNP

Model	Biological Effect	References
Regulation of TGFβ signaling		
Human CF	BNP inhibits TGFβ1-induced fibroblast proliferation and expression of fibrotic marker genes collagen 1, fibronectin, CTGF, PAI-1, and TIMP3. BNP treatment induces activation of ERK whereas chemical inhibition of ERK blocks the antifibrotic effects of BNP.	61
Mouse CF	ANP/cGMP-mediated PKG induces phosphorylation of Ser309 and Thr388 residues of Smad3, which disrupts TGFβ1-induced nuclear translocation of pSmad3 (phosphorylated at Ser423/425) and attenuates the profibrotic effects of TGFβ1.	62
Regulation of cardiac RAA system		
NRCM and NRCF co-culture	ANP/BNP reduce aldosterone synthase mRNA expression in cardiac cells, which subsequently suppresses local cardiac RAA system.	63
Cardiac <i>Npr1</i> gene deletion, MR antagonist treatment	Mice with cardiomyocyte-restricted knockdown of either NPRA or its downstream effector, PKG, develop enhanced LV hypertrophy, fibrosis, and dysfunction in response to TAC. Treating these mice with MR antagonist eplerenone, LV hypertrophy, fibrosis, dilatation, and dysfunction are attenuated. ANP signaling, mediated by NPRA and formation of cGMP, inhibits nuclear translocation of MR and thus its transcriptional activity.	64
Regulation of transcription factor GATA4		
Microarray from <i>Npr1</i> gene deleted mice	Microarray analysis of NPRA knockout LV tissue shows involvement of many factors, including calmodulin-CaMK-HDAC-Mef2 and PKC-MAPK-GATA4, in development of cardiac hypertrophy and fibrosis.	65
NRCF	ANP suppresses the expression of profibrotic ET-1 and inhibits GATA4 binding activity to ET-1 promoter. In addition, ET-1-induced GATA4 binding activity and GATA4 phosphorylation at Ser105, involved in stress-induced LV hypertrophy, are also partially inhibited by cotreatment of cells with ANP.	66
<i>Npr1</i> gene deletion	NPRA-deficient mice have enhanced calcineurin protein phosphatase activity, increased nuclear translocation of NFATc4, and increased GATA4 DNA-binding activity. Pharmacological inhibition of calcineurin suppresses both calcineurin activation and attenuates the development of cardiac hypertrophy.	67
Regulation of intracellular ion homeostasis and pH		
<i>Npr1</i> gene deletion	NPRA-deficient mice have enhanced activity of NHE-1, one of the main factors governing physiological intracellular pH in the heart. The increased activity of NHE-1 increases cellular sodium load and subsequently intracellular calcium concentration through NCX-1. Inhibition of NHE-1 by cariporide inhibits both development of cardiac hypertrophy and fibrosis. NHE-1 induction is associated with activation of CaMKII and Akt whereas adverse outcome in NPRA-deficient mice attributed to enhanced activity of MEK1-ERK1/2 and NFAT are not associated with NHE-1.	68
Isolated hearts from <i>Npr1</i> gene-deleted mice	Hearts of NPRA-deficient mice have increased expression and autophosphorylation of CaMKII, and inhibition of CaMKII inhibits development of LV hypertrophy and fibrosis.	69
NRCM, <i>Npr1</i> gene deletion × cardiac overexpression of TRPC6	ANP induces phosphorylation of TRPC6 at PKG phosphorylation site, inhibits calcium flux, and calcineurin-NFAT-dependent hypertrophy. Overexpression of TRPC6 in mice lacking NPRA exacerbates cardiac hypertrophy.	70
Regulation of cell survival kinases and regulators of GPCR signaling		
Cardiac <i>Npr1</i> gene deletion × <i>eNOS</i> gene deletion	NPRA deficiency in cardiomyocytes of hypertensive <i>eNOS</i> knockout mice leads to cardiac hypertrophy and increased fibrosis, which is accompanied by marked activation of both ERK1/2 and calcineurin.	71
Overexpression of BNP	Genetic overexpression of BNP reduces Ang II-induced ERK activation in LV.	72
Fetal rat astrocytes, NRCM, <i>Npr1</i> gene deletion × cardiac overexpression of RGS4	ANP stimulates phosphorylation of RGS4 and association of RGS4 with Gαq in vitro, whereas phosphorylation of RGS4 is reduced in hearts of NPRA knockout mice. RGS4 is required for the antihypertrophic effect of ANP in vitro; cardiomyocyte-specific overexpression of RGS4 suppresses calcineurin activity and cardiac hypertrophy in NPRA-deficient mice.	73,74
NRCM, subcutaneous administration of ANP in mice	ANP induces nuclear accumulation of zyxin and activated Akt, antagonizing apoptosis in cardiomyocytes in vitro and in vivo.	75

Ang II indicates angiotensin II; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CaMKII, Ca²⁺/calmodulin-dependent kinase II; CF, cardiac fibroblast; cGMP, cyclic guanosine monophosphate; CTGF, connective tissue growth factor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; ET-1, endothelin 1; GPCR, G-protein-coupled receptor; Gαq, guanine nucleotide-binding protein (G protein) αq subunit; HDAC, histone deacetylase; LV, left ventricle; MAPK, mitogen-activated protein kinase; MR, mineralocorticoid receptor; NCX-1, sodium-calcium exchanger 1; NFAT, nuclear factor of activated T-cells cytoplasmic 4 isoform; NHE-1, sodium hydrogen antiporter 1; NPRA, natriuretic peptide receptor A; NRCF, neonatal rat cardiac fibroblast; NRCM, neonatal rat cardiomyocyte; PAI-1, plasminogen activator inhibitor 1; PKC, protein kinase C; PKG, protein kinase G; RAA, renin-angiotensin-aldosterone system; RGS4, regulator of G protein signaling subtype 4; Ser, serine; TAC, transverse aortic constriction; TGFβ1, transforming growth factor beta 1; Thr, threonine; TIMP3, tissue inhibitor of metalloproteinase 3; TRPC6, transient receptor potential canonical channel 6.

However, in preparations from failing mouse heart, the response elicited by CNP is 2-fold compared to the response of ANP, thus suggesting marked reduction in NPRA activity, but no change in NPRB activity.⁷⁶ CNP levels are substantially increased in myocardial tissue in patients with HF.⁷⁷ However, plasma levels of CNP are very low even in patients with severe HF. Levels of circulating CNP actually appear to decrease in rats during aging, and this correlates with an increase in cardiac fibrosis.⁷⁸ In addition to the heart, head and neck tissue are important sources of CNP.⁷⁹

In vivo cleavage of proCNP by furin yields 50 amino acid amino terminal proCNP (NT-proCNP) and CNP1-53, which is the precursor for the biologically active mature form of CNP (CNP1-22). Administration of CNP in vitro has been shown to exert antihypertrophic effects on cardiomyocytes as well as antiproliferative effects on rat vascular smooth muscle cells and primary fibroblasts.⁸⁰ Of the three NPs, CNP has been shown to be most potent at inhibiting growth-factor-induced smooth muscle cell migration.⁸¹ CNP has only minimal renal actions, but it has been shown to induce vasorelaxation and, interestingly, CNP infusion to dogs has been shown to lower blood pressure more effectively than ANP without natriuretic effect.⁸² Plasma levels of CNP1-53 are also elevated in patients with acute decompensated HF,⁸³ and recent data suggest that in addition to CNP1-22, the CNP1-53 peptide also has biological actions in in vitro and in vivo models.⁸⁴ Apart from regulating cardiovascular functions, disruption of CNP gene in mice results in impaired longitudinal growth of long bones and dwarfism.⁸⁵ Mutations in the NPRB gene (*NPR2*) in humans impairs skeletal growth, and mice with targeted deletion of *Npr2* show impaired endochondral ossification, defective development of female reproductive organs, and early mortality.⁸⁶

Blood pressure analysis of mice with *Npr2* deletion revealed no difference compared to wild-type littermates. Fibroblasts from NPRB-deficient mice, however, showed blunted cGMP elevation in response to CNP stimulation.⁸⁶ Overexpression of dominant-negative NPRB in rats led to progressive cardiac hypertrophy that was not owing to elevation in blood pressure.⁸⁷ On the other hand, continuous infusion of CNP for 2 weeks in rats with experimental MI significantly reduced both cardiac hypertrophy and fibrosis.⁸⁸ Furthermore, transgenic overexpression of CNP in cardiomyocytes has been shown to prevent development of LV hypertrophy post-MI.⁸⁹ The cGMP-dependent signaling appears to be central for antifibrotic effects of CNP.^{88,90} Evidence from Burnett's laboratory suggests that a non-cGMP-dependent pathway also mediates the antifibrotic effects of CNP.⁷⁸ In conclusion, antifibrotic and -hypertrophic effects of CNP appear to be mediated both by activation of NPRB and by a non-cGMP-dependent mechanism, whereas blood-pressure-lowering effect of CNP is not dependent on activation of NPRB.

Natriuretic Peptides in the Regulation of Energy Metabolism, Development of Metabolic Syndrome, and Diabetes

The energy needed to maintain cardiac contractility is mainly produced by fatty acid oxidation in mitochondria, the organelles that are highly abundant in heart tissue. However, if needed or when available, the heart is able to use other energy substrates, such as carbohydrates, lipids, amino acids, and ketone bodies, for adenosine triphosphate (ATP) production.⁹¹ In pathological hypertrophy, cardiac metabolism is characterized by reduced fatty acid oxidation and increased glucose uptake and glycolysis. On the other hand, in diabetes, insulin resistance limits glucose entry into cardiomyocytes to be metabolized for energy. In diabetes, fatty acid oxidation is increased and associated with accumulation of lipids and increased consumption of oxygen. These metabolic derangements lead to impaired cardiac function. However, the situation is quite complex—no matter how beneficial enhanced glucose use is in cardiac events, promotion of fatty acid oxidation would be rather desirable for long-term source of energy production.

In addition to their chief function as cardiovascular hormones, NPs have been shown to contribute to regulation of energy metabolism. ANP and BNP serve as potent mediators of lipolysis in adipocytes, whereas CNP has only a minor lipolytic effect.⁹² Insulin is known to counteract the lipolytic effect induced by catecholamines in sympathetic activity by counteracting the cyclic adenosine monophosphate (cAMP)/protein kinase A–signaling pathway. However, insulin has no effect on ANP-induced lipolysis in adipocytes. Lipolysis induced by NPs is likely to occur by a cGMP-dependent mechanism and to be independent of the activation of sympathetic nervous system and cAMP signaling.⁹³ When infused into the circulation or administered into subcutaneous adipose tissue in humans, ANP leads to lipid mobilization. Whereas endurance training improves lipid mobilization, regulated by catecholamines and insulin, exercise also induces release of NPs from the heart, which contribute to lipid mobilization concomitantly with sympathetic activation. NPs have also been shown to increase mitochondrial oxidative metabolism and lipid oxidation in skeletal muscle, leading to enhanced energy metabolism in muscle.⁹⁴

Opposite to white adipose tissue as a fat storage, brown adipose tissue is thermogenic by producing heat while dissipating energy. The physiological importance of brown adipose tissue in the regulation of energy metabolism in adults has recently emerged (reviewed in previous work⁹⁵). Interestingly, NPs may regulate brown adipose tissue and activate its thermogenic activity while controlling the status of white adipose tissue. NPs can promote “browning” of white

adipocytes by upregulating mitochondrial uncoupling receptor 1 and peroxisome proliferator-activated receptor (PPAR)- γ coactivator (PGC)-1 α , leading to increased mitochondrial biogenesis and uncoupled respiration similarly in response to β -adrenergic sympathetic activity, in a p38 MAPK-dependent manner.⁹⁶

In addition to increased insulin sensitivity, achieved by alterations in adipose tissue and energy expenditure of skeletal muscle, NPs may also increase insulin secretion. When studied in a mouse model, ANP was found to block ATP-dependent potassium channel activity in pancreatic β -cells, to increase glucose-elicited calcium signaling and enhance glucose-stimulated insulin secretion in islets of Langerhans.⁹⁷ It should be noted, however, that long-term exposure to ANP may inhibit glucose-stimulated insulin secretion from isolated islets, which is accompanied with reduced ATP generation in β -cells in response to glucose.⁹⁸

To pinpoint the importance of natriuretic peptides as metabolic regulators, BNP-overexpressing mice which were fed on high-fat diet were protected against diet-induced obesity and insulin resistance.⁹⁹ This was associated with increased muscle mitochondrial biogenesis and fat oxidation through upregulation of PPAR- γ coactivator PGC-1 α and PPAR- δ . Furthermore, chronic BNP treatment at low dose reduced plasma glucose levels, improved metabolic profile, and prevented development of myocardial dysfunction in obese diabetic mice.¹⁰⁰ When BNP levels were determined from HF patients, obese patients indeed had reduced BNP plasma levels, although no difference in severity of HF or proinflammatory cytokine levels were detected.¹⁰¹

In humans, metabolic risk factors and components of metabolic syndrome were found to be associated with low natriuretic peptide levels in plasma.¹⁰² In a prospective study, low plasma levels of ANP were found to predict development of future diabetes, suggesting a causal role of ANP deficiency in diabetes development.¹⁰³ In another study, higher NP levels were associated with a favorable body-fat profile, including lower visceral fat and reduced insulin resistance.¹⁰⁴ In a follow-up study, higher NP levels were associated with reduced risk for diabetes.¹⁰⁵

NPs can also modulate secretion of adipokines, such as adiponectin and leptin, from adipose tissue and secretion of cytokines from adipose tissue macrophages, as studied in vitro. NPs may thus participate in regulating inflammatory status in obesity. Some of the favorable metabolic effects of NPs may even be mediated by their anti-inflammatory and hepatoprotective effects in liver.⁹³ CNP, mainly expressed in the brain, might participate in regulation of energy metabolism by controlling food intake and satiety. Various actions of NPs in energy metabolism have been recently comprehensively reviewed.¹⁰⁶ Although there are extensive data showing NPs to play a role in controlling energy metabolism, the extent

of their contribution and necessity to metabolic events remains to be elucidated.

In summary, aside from their classic hemodynamic effects, NPs have been associated with regulation of numerous physiological functions controlling energy metabolism, as summarized in Figure 2. NPs may activate lipolysis, lipid oxidation, and mitochondrial respiration and promote white adipose tissue browning, increase muscular oxidative capacity, and protect against diet-induced obesity and insulin resistance. These metabolic events are, in turn, closely associated with development of cardiovascular diseases. By secretion of NPs, the heart may thus play a central role in regulation of energy balance.

Novel Therapeutic Approaches

Recombinant ANP (carperitide) was first approved for treatment of patients with acute decompensated congestive HF in Japan in 1995. Recombinant BNP (nesiritide) has been approved by the U.S. Food and Drug Administration (FDA) for the same indication in 2001. However, data from the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure Trial (ASCEND-HF) showed a lack of efficacy on rehospitalization for HF or death from any cause—the major clinical endpoints.¹⁰⁷ In a pilot study conducted with 40 patients with New York Heart Association functional class II to III HF, indicating slight to marked limitation in activity owing to cardiovascular symptoms, chronic protein therapy with subcutaneous BNP was sufficient to improve Minnesota Living with Heart Failure score, LV remodeling, and LV filling pressure.¹⁰⁸

Novel therapeutic approaches include oral delivery of conjugated BNP, which activated cGMP and reduced mean arterial pressure in a model of acute Ang II-induced hypertension.⁴¹ More recently, cenderitide, a chimeric peptide that simultaneously activates NPRA and NPRB, has entered clinical trials for preservation of LV function post-MI. Cenderitide has also been shown to be more resistant to degradation by neutral endopeptidase neprilysin compared to ANP, BNP, and CNP.¹⁰⁹ ProBNP has been shown to have 7 O-glycosylation sites in the NT-proBNP region and furin is effective in cleaving deglycosylated, but not intact proBNP.^{110,111} A subsequent study showed that O-glycosylation of T71 residue in proBNP attenuates its processing into active BNP.¹¹² Nonglycosylated proBNP is processed into active BNP1-32 in plasma, but the processing is delayed in plasma samples from patients with HF.¹¹³ When compared to healthy controls, the processed BNP is degraded more rapidly in HF with preserved ejection fraction whereas the processing is delayed in HF with reduced ejection fraction.¹¹³ Characterization of the regulatory mechanisms of NP processing and degradation is

thus crucial and may offer novel therapeutic options for treatment of HF.

Neutral endopeptidase neprilysin is a membrane-bound endopeptidase that is widely expressed in mammals, including the kidney, lung, endothelial cells, vascular smooth muscle cells, cardiomyocytes, fibroblasts, adipocytes, and the brain.⁸ Neprilysin has been shown to degrade several vasoactive peptides, including natriuretic peptides, bradykinin, and adrenomedullin. Candoxatril is a neprilysin inhibitor developed for clinical use. In patients with HF, candoxatril increased ANP and BNP levels, but had unfavorable vascular and cardiac effects.¹¹⁴ Combining neprilysin inhibition with inhibition of angiotensin-converting enzyme (ACE) showed beneficial effects in experimental models. However, clinical trials with omapatrilat, which inhibits both neprilysin and ACE, proved unsuccessful owing to serious angioedema.¹¹⁵ A plausible explanation for the increased incidence of angioedema is inhibition of breakdown of bradykinin and aminopeptidase P by omapatrilat.

Angiotensin receptor blockers (ARBs) have a lower risk of angioedema compared to ACE inhibitors, and combining neprilysin inhibition with angiotensin receptor blockade was shown to have an additive blood-pressure-lowering effect compared to valsartan alone without the increased incidence of angioedema.¹¹⁶ The Paradigm-HF study was conducted to compare the efficacy of combined angiotensin receptor blockade and neprilysin inhibition by valsartan/sacubitril (previously known as LCZ696) to that of enalapril in patients with chronic HF and reduced ejection fraction. The trial was stopped early because of the overwhelming benefit of LCZ696. Combined angiotensin receptor blockade and neprilysin inhibition by LCZ696 significantly reduced the risk of hospitalization for HF and the number of deaths for cardiovascular causes.¹¹⁷ Importantly, no major safety concerns of the combination emerged during the study. It should be noted, however, that the LCZ696 group developed significantly more cases of hypotension, which was already applied as exclusion criteria. This raises a concern of hypotension as a potential adverse effect with not just LCZ696, but possibly also with other NP-enhancing drugs. Simultaneous blockade of angiotensin receptors and neprilysin by LCZ696 has also shown efficacy in an experimental model attenuating cardiac remodeling and dysfunction in rats post-MI.¹¹⁸ The FDA has approved this drug, valsartan/sacubitril, as a treatment for HF in July 2015.

Given that higher NP levels have been shown to associate with a favorable body-fat profile and with reduced risk for diabetes, it will be of interest to assess whether neprilysin inhibition is also sufficient to affect energy metabolism. Some concerns have been raised over possible risk for Alzheimer's disease (AD) resulting from neprilysin inhibition.¹¹⁹ The brain neprilysin has been shown to play a key role in degrading

β -amyloid, of which aggregation into plaques is one of the hallmarks of AD.¹²⁰ It is not likely that peripheral inhibition of neprilysin affected brain β -amyloid levels by inhibiting the peripheral sink effect, which is suggested as an alternative for β -amyloid removal.^{121,122} However, if LCZ696 was to penetrate the blood-brain barrier, it could, in theory, accelerate the progression of AD by reducing β -amyloid degradation in brain. On the other hand, vascular diseases, such as hypertension, are known risk factors for the progression of AD, and an ARB component of LCZ696 could therefore attenuate the progression of AD. Notably, dementia-related adverse effects were not increased in the Paradigm-HF trial. The trial, however, was not designed for evaluation of the cognitive function, and follow-up studies are needed to address possible adverse effects on cognitive function.

Another novel drug in the group of NP-modifying molecules is ularitide, which is the chemically synthesized form of the human NP, urodilatin. Urodilatin is produced in humans by alternative processing of proANP in distal renal tubule cells and it participates in sodium homeostasis. Ularitide causes vasodilation, diuresis, and natriuresis by binding to NPRA and activation of the cGMP pathway. Ularitide is currently in a phase III clinical study, called TRUE-AHF, to study its efficacy and safety in treatment of HF.¹²³ Ularitide actually shares many pharmacological properties with the recombinant BNP-analog, nesiritide.

Perspectives

NPs function to maintain normal salt and water balance and play a critical role in regulating arterial blood pressure through their natriuretic, diuretic, and vasodilator effects. ANP and BNP are well-established markers of both acute and chronic HF, and the circulating levels of NPs have been shown to correspond to the degree of LV dysfunction.¹²⁴ In addition, NPs provide long-term prognostic value in HF patients and in those with acute cardiac ischemia.^{125,126} There is also evidence that levels of circulating NP provide predictive value in the healthy population.¹²⁷ There is now compelling evidence showing that NPs are also important regulators of cardiac structure and function. Especially, it appears that NPs are key physiological antagonists of the cardiac RAA system. These findings may provide novel indications for therapy with NPs and their conjugates. In addition, the favorable effects of NPs on cardiac structure should be considered in the design of novel HF therapies. Countering the detrimental changes in expression of genes regulating cardiac hypertrophy, fibrosis, and calcium handling is central in preventing adverse structural and functional myocardial remodeling. Therefore, it may be a desirable goal for HF therapy to preserve the elevated levels of natriuretic peptides.

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Disclosures

None.

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