Atrial Natriuretic Peptide
— Old But New Therapeutic in Cardiovascular Diseases —

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With the discovery of atrial natriuretic peptide (ANP), the heart as an endocrine organ was established. Basic science revealed that ANP, through the particulate guanylyl cyclase A receptor and cGMP, plays a fundamental role in cardiorenal biology. This work has led to the development of ANP as a therapeutic, especially in heart failure (HF). Human genomics has strengthened our understanding of ANP, revealing specific ANP gene variants that may be associated with biological dysfunction, but also may mediate protective properties, including in metabolic syndrome. Advances in understanding the processing and degradation of ANP molecular forms have resulted in therapeutic breakthroughs, especially inhibition of ANP degradation by neprilysin inhibitors. Although ANP is administered intravenously for acute HF, a novel therapeutic strategy is its chronic delivery by subcutaneous injection. An innovative therapeutic development is engineering to develop ANP-based peptides for chronic use. These interconnected topics of ANP biology and therapeutics will be reviewed in detail.

Key Words: Atrial natriuretic peptide; Cardiorenal biology; cGMP

In 1964, Eugene Braunwald first proposed that the heart was not only a pump, but also an endocrine organ, secreting neurohormonal factors with biological actions on the heart and elsewhere. These secreted neurohormonal factors have proven important for the clinical diagnosis and therapy of heart disease, as well as for cardiovascular research. Because of this research, we now have a more sophisticated view of heart failure (HF) as a neurohormonal disorder and the use of neurohormonal therapeutics is an excellent example of reciprocity in translational science.

Among the neurohormonal factors excreted by the heart is a family of peptides known as the natriuretic peptides (NPs). The original member of this family is a cardiorenal protective hormone, atrial NP (ANP) discovered by Drs. de Bold and Kangawa. ANP possesses diuretic, natriuretic and vasodilatative properties, suggesting ANP may be an efficacious therapeutic for HF. Indeed, recombinant human ANP, called carperitide, has been used successfully in Japan for the treatment of acute HF, proving safe and effective in the COMPASS trial and improving the prognosis in the PROTECT trial. Because ANP has several pleiotropic effects, therapeutic opportunities beyond acute HF may exist. In this review we will focus on the therapeutic challenges and opportunities for ANP-related peptides in cardiovascular diseases (CVD) and metabolic syndrome (MetS).

Physiology of ANP
The mature 28-amino acid (AA) form of ANP has biological actions via its receptor, particulate guanylyl cyclase (pGC) receptor-A (GC-A), which activates cGMP generation. Disruption of NPs and/or their receptors disturbs the balance of the NP system, with physiological consequences. Studies have helped us to better understand the role of the NP system in cardiovascular homeostasis. Wang et al reported that ANP null mice developed cardiac hypertrophy, exhibited an exaggerated hypertrophic response to pressure overload by transverse aortic constriction (TAC), and showed increased expression of extracellular matrix proteins after TAC compared with wildtype (WT) mice. In addition, Li et al reported that left ventricular (LV) hypertrophy and fibrosis were exaggerated in response to pressure overload by TAC in ANP null mice compared with WT mice. These studies underscored the link between the heart and kidney in which the ANP via GC-A plays a fundamental role in sodium homeostasis and renal structure.

Human Genetic Variants of ANP
Figure 1 illustrates the location of the human NPPA gene on chromosome 1. From this gene a 151 AA preproANP is
produced by gene transcription, which is then processed to a 126-AA proANP after removal of the signal peptide (Figure 1).\textsuperscript{14,15} Our understanding of ANP genetic variants was initially introduced by Newton-Cheh et al in 2009.\textsuperscript{16} There are 3 variants that significantly relate to cardiovascular phenotypes and also a frameshift mutation of ANP (MANP), all located in the NPPA gene. These variants modulate the production and/or function of ANP, influencing cardiovascular function and/or metabolic phenotypes.

**rs5065 (TC2238)** and **rs5063 (V32M)**

rs5065 is a stop codon mutation in exon 3 (Figure 1), responsible for the synthesis of 2 extra AAs (Arg-Arg) on the c-terminal end of mature 28AA ANP,\textsuperscript{17} which is also called ANP-RR. Surprisingly, the minor allele with only 2 additional AAs affects cardiovascular profiles. Rubattu et al\textsuperscript{18} studied the rs5065 variant in 206 patients with ischemic stroke compared with 236 controls in Italy. The frequency of the minor allele was significantly higher in ischemic stroke patients than in the controls. The recurrence of stroke was also significantly higher in the minor allele cases compared with the major allele. In a US study of 1,810 coronary disease patients, Ellis et al reported that the minor rs5065 allele was associated with lesser history of hypertension (HT) and a reduced rate of cardiovascular readmission.\textsuperscript{19} In a European study, Barbato et al showed that in 1,004 patients with the rs5065 gene undergoing coronary angiography, the minor allele was an independent predictor of acute coronary syndrome and a higher incidence of major adverse cardiac events in patients with stable angina compared with controls.\textsuperscript{20} Finally, in 1,623 general population patients in Olmsted County, Minnesota, the minor allele was associated with an increased risk of stroke and a higher prevalence of myocardial infarction (MI).\textsuperscript{21} However, none of clinical studies showed changes in circulating levels of ANP associated with the minor allele. These studies suggested that the rs5065 minor allele may have an association with atherosclerotic phenotypes such as stroke and coronary artery disease.

In an attempt to understand the mechanism behind rs5065/ANP-RR, we confirmed that ANP-RR stimulated cGMP production, similar to ANP, but enhanced endothelial permeability more than ANP in endothelial cells.\textsuperscript{21} Other investigators reported that ANP-RR reduced cell viability and increased apoptosis, necrosis and oxidative stress in vascular smooth muscle cells (VSMCs),\textsuperscript{22} and reduced cell survival and endothelial tube formation while increasing cell apoptosis in endothelial cells via greater binding to NPR-C than ANP.\textsuperscript{23} Interestingly, ANP-RR reduced apoipoprotein E (ApoE) gene expression via NPR-C in VSMCs, which suggests NPR-C stimulation by ANP-RR interferes with the vasoprotective actions of ApoE.\textsuperscript{24} These findings suggest that the rs5065 minor allele may produce a “malignant” ANP for the endothelium leading to atherosclerotic diseases through a NPR-C pathway. Therefore NPR-C inhibition may be a seminal therapeutic target for subjects with rs5065 to attenuate the risk of atherosclerotic diseases.

The minor allele of rs5063 variant is responsible for changing the 32nd AA of preproANP (7th AA of long-acting NP (LANP)) (Figure 1). Pereira et al genotyped 16,000 genes in 891 subjects from the general community to identify the genetic determinants for plasma NT-proANP.\textsuperscript{25} In the 33 genome-wide significant single-nucleotide polymorphisms (SNPs) that were identified in the MTHFR-CLCN6-NPPA-NPPB locus, the minor allele of rs5063 represented the most significant variation for lower NT-proANP levels together with higher blood pressure (BP), and an increased risk of stroke. In vitro study showed that the rs5063 variant allozyme in transfected HEK293 cells was decreased to 55±8% of WT protein, which may contribute to the lower levels of NT-proANP.

**rs5068 and mir-425**

In contrast to rs5063 or rs5065, another known ANP gene variant may be a “good friend” for MetS and/or HT. In 2009, Newton-Cheh et al identified the rs5068 genetic variant, which was associated with an increase in plasma ANP levels.\textsuperscript{16} The minor rs5068 allele was associated with lower BP, as well as reduced odds of HT in 14,743 individuals of
ANP Molecular Forms, Processing and Degradation

The molecular precursor of ANP, 126AA proANP, is formed by the removal of the signal peptide from preproANP (Figure 1). ProANP is stored in secretory granules in atrial cardiomyocytes and, when needed, is cleaved and released, as in response to stretch. Figure 2 illustrates proANP processing into ANP molecular forms. ProANP is processed into NT-proANP and ANP by the cardiac serine protease corin. ProANP is also processed into urodilatin in the kidney by an unknown enzyme. NT-proANP can be further processed into 3 fragments: LANP, vessel dilator, and kaliuretic peptide. Inactivation of ANP is achieved by NPR-C binding, which clears the peptide by endocytosis, or by enzymatic degradation by neprilysin (NEP) and insulin-degrading enzyme (IDE) (Figure 3). The inactivation process for other ANP molecular forms is unknown.

Taking into consideration the metabolic processes of ANP, possible therapeutic targets include the following: (1) exogenous treatment with active ANP molecular forms, (2) modulation of proANP processing by corin, or (3) inhibition of ANP degradation. The therapeutic opportunities of these 3 strategies are discussed next.

Exogenous Treatment With Active ANP Molecular Forms

There are 2 mechanisms to increase ANP levels in the body: exogenous administration of active molecular forms, and ANP gene delivery to increase local expression. With regard to exogenous administration, carperitide and ularitide (urodilatin) have been used in the clinical setting for acute HF. Unfortunately, although effectiveness in acute HF with ularitide was expected, as with carperitide, the Phase III trial (TRUE-AHF) while showing benefit during acute administration did not show better long-term outcomes compared with placebo. Other molecular forms of ANP (i.e., LANP, vessel dilator, and kaliuretic peptide (Figure 2)) have also been tested in humans. Vesely et al reported that all 3 peptides have more urinary effects than ANP in human HF, but no large clinical trials have been done. We have reported that full-length proANP is biologically active in vitro and in vivo in normal canines, however, no human
studies have been done because of the high cost and difficulties associated with large peptide synthesis for clinical use. Clinically, it would be challenging to administer peptides chronically because it is technically difficult to develop oral peptides, and subcutaneous (SQ) ANP does not absorb well into the circulation because there is SQ degradation. This is one of the reasons why NPs have only been used in acute HF where it can be administered by intravenous infusion.

**ANP Gene Delivery**

Gene therapy using NPs for HF has been in development for more than 2 decades, but the choice of gene and delivery system used is critical. Most recently, AAV-based vectors emerged as promising gene delivery vehicles. Based on the early promise of a small dose-finding study with an AAV1 vector expressing SERCA2a (CUPID), a Phase 2b trial (CUPID2) was conducted but the results did not achieve the set endpoints, although no safety issues were observed.

Cataliotti et al in our group reported that gene delivery using the other GC-A activator, B-type NP (BNP), in hypertensive rats resulted in lower cardiac hypertrophy and better cardiac function and prognosis. In the 1990s, ANP gene delivery was tested in preclinical studies. Adeno-virus harboring human ANP was administered via single intravenous injection in Dahl salt-sensitive rats. Immunoreactive ANP was detected in the heart, lung, kidney, and brain of the rats after the delivery. ANP gene delivery attenuated HT, cardiac hypertrophy, and renal injury, as well as decreased the stroke death rate and the thickness of the aortic wall. Therefore, ANP gene delivery has therapeutic potential for HT and possibly other cardiac and renal disease states, but further studies are warranted.

**Corin-Mediated Processing**

Currently, corin is the only enzyme known to process proANP and it is present in the heart, kidney, and the circulation. Chan et al reported corin-deficient mice (Cor−/−) had elevated proANP levels and undetectable ANP, together with spontaneous HT and cardiac hypertrophy. Studies of corin SNPs revealed that the corin minor allele was associated with higher BP and LV hypertrophy, and an increased risk for prevalent HT. Tripathi et al reported that decreased corin levels promoted the transition from the early to late stage of HF with impaired proANP processing in a dilated cardiomyopathy (DCM) model in mice. When considered together, these studies suggest that corin may play a role in controlling the HT and cardiac hypertrophy that proceed to the development of HF, specifically through proANP processing.

There are only a few studies suggesting possible therapeutic strategies regarding corin to facilitate proANP processing to ANP. Gladysheva et al reported that cardiac corin overexpression in DCM mice resulted in reduced cardiac fibrosis, and improved cardiac function, HF and prognosis. The results suggested that corin gene delivery to the heart may improve cardiac function and HF. A second therapeutic approach could involve increasing corin activity. Chen et al reported the enzyme PCSK6 can activate corin activity, but it has not been studied therapeutically. A third approach would be administration of active corin enzyme, but no studies have been reported. We speculate that it may be challenging to synthesize “active” corin for in vivo studies.

**Inhibition of ANP Degradation**

ANP is inactivated via binding to the clearance receptor NPR-C or by enzymatic degradation by NEP or IDE (Figure 3). Increasing endogenous ANP levels (i.e., prolonging the half-life of ANP) could therefore be achieved by inhibition of NPR-C and/or by reducing NEP/IDE activities.

Historically, NPR-C has been thought of as a clearance receptor of NPs, but important functions have now been revealed. In NPR-C KO mice, homozygotes survived birth, but 50% died before weaning. The surviving homozygotes had skeletal deformations associated with a considerable increase in bone turnover. The half-life of ANP in the circulation of homozygotes lacking NPR-C was two-thirds longer than in the WT; therefore, NPR-C has some important functions beyond clearing ANP. Some reports suggest that NPR-C has roles in the L-type Ca2+ current in myocytes and sino-arterial nodes together, and to inhibit proliferation of cardiac fibroblasts with BNP. On the other hand, it may be strategic to inhibit NPR-C because ANP-RR may mediate vascular injury via NPR-C, as we stated before, because NPR-C seems to exhibit “pleiotropic”...
effects other than clearance, it may be controversial to therapeutically inhibit NPR-C just to prolong ANP’s half-life. Because ANP is known to be rapidly degraded by NEP, inhibition of NEP may be a good therapeutic target. In the 1990s, there were preclinical and clinical studies for dual inhibition of angiotensin-converting enzyme (ACE) and NEP using a novel small molecule, omapatrilat. Omapatrilat increased circulating ANP and cGMP levels and increased urinary sodium and water excretion in clinical trials. Also, omapatrilat reduced aortic leakiness and atheroma formation with enhanced endothelial-independent vasorelaxation of ANP in a rabbit model of atherosclerosis, suggesting a therapeutic opportunity in HT and other atherosclerotic diseases. Although clinical trials of omapatrilat in HF (OVERTURE) and in HT (OCTAVE) showed improved clinical status compared with ACE inhibition alone, its approval was declined in the USA in 2000 because of a greater risk for angioedema. A second-generation dual ACE/NEP inhibitor, LCZ696 or Entresto (sacubitril/valsartan, Novartis), has now been approved for use in the clinic for chronic augmentation of the endogenous NP/cGMP system, which showed positive results in chronic HF with reduced ejection fraction (HFrEF). Specifically, Entresto inhibited the degradation of endogenous NPs (increased BNP levels), together with increased plasma cGMP levels, decreased levels of NT-proBNP, and reduced risk of death and rehospitalization in HFrEF patients compared with enalapril in the successful Phase III PARADIGM-HF trial. Although not measured, we assume Entresto also increased endogenous ANP levels similar to omapatrilat.

The other potential target is inhibition of IDE. Muller et al reported that ANP is degraded by IDE in rats. Rałat et al reported that IDE rapidly cleaves ANP, so inhibition of IDE expression may enhance the action of GC-A by prolonging the half-life of ANP. Inhibition of IDE in CVD has not been investigated yet, but IDE inhibition may be a good therapeutic target for type II diabetes and Alzheimer’s disease, as has been reported. For example, Maiani et al performed a chemical and biochemical survey of a DNA-template library and found an optimal small-molecule inhibitor, 6bk IDE inhibitor. Acute administration of the 6bk IDE inhibitor resulted in the improvement of glucose tolerance in obese rats, suggesting the IDE inhibitor may be a potential therapeutic for CVD, especially when complicated with type II diabetes.

**ANP Delivery**

Given the neutral results of the nesiritide (BNP) and ularitide Phase III trials for acute HF, and the “success” of chronic administration of Entresto, we hypothesized that long-term and relatively low-dose administration of ANP may be an optimal therapeutic strategy for cardiovascular and metabolic diseases. Carperitide has been successfully used in Japan for relatively longer periods of time (up to 7 days) compared with nesiritide or ularitide, which were administered for up to 48 h. In the past 2 decades, we have developed novel chronic delivery systems for NPs in CVD. We reported that chronic delivery of BNP by innovative oral delivery in experimental HT had beneficial actions. We also reported that 8 weeks of daily SQ BNP reduced LV hypertrophy, improved myocardial function, maintained the glomerular filtration rate (GFR), suppressed renin and improved symptoms. As oral peptide compounds are markedly expensive, the clinical use of oral administration is not yet practical. Therefore, chronic SQ delivery of ANP for MetS and HT may be the optimal strategy. However, because SQ ANP is easily degraded and not well absorbed into the circulation, we have developed a novel ANP/GC-A/cGMP-based peptide for SQ delivery.

**MANP: a New Generation of ANP-Based Therapy in HT**

**Discovery and Preclinical Studies**

MANP (Figure 4) is a 40-AA NP containing 12 additional AA on the C-terminus of ANP, found as a NPPA gene mutation in exon 3 (Figure 1). The significant biological differences between ANP and MANP is prolonged proteolytic degradation by NEP. We confirmed that intravenous continuous infusion of MANP resulted in significantly greater cGMP activation, diuretic, natriuretic, GFR-enhancing, RAAS-inhibiting, cardiac-unloading, and BP-lowering effects compared with an equimolar dose infusion of ANP in vivo in normal canines. MANP infusion also resulted in significant BP-lowering effects, cardiac unloading, and enhanced renal function compared with placebo in an acute HT canine model. Therefore, these results support that MANP may be a potential therapeutic for HT.

Resistant HT (RH) is the most severe form of HT, with high morbidity and mortality, and for which there are no approved drugs or devices. Further, the mechanisms of RH, such as sodium retention with intravascular volume expansion, potential vasoconstriction and hyperaldosteronism, are all possible targets for MANP. Therefore, it is important to note that MANP has moved from design, preclinical in vitro and in vivo investigations, to an investigational new drug, and a first-in-human clinical trial for RH.

**Phase I Clinical Trial for HT**

We have recently completed a Phase 1, 2-part, first-in-human trial for safety, and determination of the maximum tolerated dose (MTD) with SQ administration of MANP in hypertensive subjects. Part A was an open-label, sequential, single-ascending dose design study (1, 2.5 and 5 µg/kg) with 3 cohorts of 4 stable HT subjects. The MTD was 5 µg/kg, which resulted in maximal systolic and diastolic BP reductions of 20±18 and 12±5mmHg, respectively.

Part B was a randomized, double-blind, placebo-controlled design in 4 cohorts of 5 subjects with resistant “like” HT. The patients had to be on at least 3 medications and with BP >145/70mmHg. Four cohorts were dosed at 0.5, 2.5, 3 and 5 µg/kg, respectively, and the MTD in Part B was...
also 5μg/kg, which resulted in sustained decrease of BP for 24h (max. reduction systolic BP: MANP, 26±14 vs. placebo, 1±12mmHg) with reductions in aldosterone levels.

Because MANP was well tolerated without serious adverse events, single SQ administration in stable HT or resistant-like HT was safe and highly effective in lowering BP with events, single SQ administration in stable HT or resistant-like HT was safe and highly effective in lowering BP with events.

**Future Directions**

The future of the ANP biology will move in several directions. One is to better understand the regulation of ANP production in the heart. Already it is clear from human genomics that a key microRNA may regulate ANP production. This knowledge offers new ways to enhance production as a novel therapeutic strategy. As beneficial metabolic actions of ANP are revealed, ANP and ANP-based therapeutics may represent therapies for diabetes, obesity and MetS. Finally, novel peptide engineering/delivery strategy will be a new frontier for the development of innovative therapeutics for CVD.

**Conclusions**

Study of ANP has given us great insights into the heart as an endocrine organ, as well as better understanding the GC-A and GCMP-signaling pathways. Both preclinical and human genomics have established that ANP now plays a fundamental role in cardiorenal and metabolic homeostasis. Advances will continue to occur in which ANP and ANP-based peptides will be developed to have wide therapeutic applications for CVD.

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**Relationship With Industry**

J.C.B Jr is Co-Founder of Zumbo Discovery and holds equity.

**References**


