Project thesis

*Natriuretic peptides as therapeutic agents*

A literature review

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Natriuretic peptides as therapeutic agents – a literature review

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Abstract

The natriuretic peptide system (NPS) consists of a group of autocrine, paracrine and endocrine factors that mediate a diverse array of biological effects that indicate that there may be a possible therapeutic role for agents augmenting this system in many forms of disease, especially heart failure (HF). Numerous researchers have investigated the NPS since it was first described nearly 40 years ago. Several therapeutic agents have been developed; all represent different natriuretic peptide (NP)-augmenting strategies and can be classified as either recombinant natriuretic peptides (NPs), designer NPs or inhibitors of NP degradation.

Two recombinant forms of NPs (Carperitide, Nesiritide) were approved for treatment of acute decompensated HF in Japan and the USA in 1995 and 2001, respectively. However, disappointing results from more recent, larger clinical trials will probably put an end to further use of these agents.

Other attempts to exploit the NPS therapeutically include the use of Neprilysin inhibitors (NEP-I), a group of agents that inhibit the key enzyme responsible for NP degradation (Neprilysin), Vasopeptidase inhibitors (aka dual ACE/NEP-I), angiotensin receptor neprilysin inhibitors (ARNI) and so-called designer NPs: engineered peptides containing different segments of native NPs. Designer NPs are still in the early stages of development, but have shown encouraging results in “proof-of-concept”-studies, prompting further investigations.

LCZ696 (Entresto; Sacubitril/Valsartan) is a first-in-class ARNI and probably the most successful therapeutic agent targeting the NPS. It was approved for treatment of selected HF patients in 2015. In this thesis, we will review these agents in more detail, including their history, current status and possible role in future treatment of cardiovascular and other diseases. We will discuss the historical background of these agents and their possible role in future treatment of cardiovascular (CV) and other diseases.
Preface

Before I started writing my project thesis, I attended the medical student research program. One of the projects in the group I was working with tried to develop novel, small-molecular, non-peptide natriuretic peptide receptor (NPR) agonists and/or positive allosteric modulators. Due to illness, I had to resign before I was able to complete the program. Nevertheless, my interest in this field of research has not diminished. For this reason, I chose to write my project thesis on natriuretic peptides as therapeutic agents.

The aim of my project thesis is to write an updated review on the past, present and future approaches to the use of natriuretic peptides as therapeutic agents. In my thesis, I want to answer the following questions; which therapeutic agents targeting the natriuretic peptide system (NPS) have been developed? Which studies have investigated the NPS as a therapeutic target and what were their results? What were the reasons for the success or failure of natriuretic peptides (NPs) as therapeutic agents?

To answer these questions, I decided that a literature review would be the best approach. A systematic review and meta-analysis would have been optimal, but this was not feasible considering the time at my disposal. Therefore, I chose to write a literature review. In my thesis, I will give a historical background and then discuss past, present and future approaches to NPs as therapeutic agents.
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1 Introduction

1.1 Historical background

Two studies conducted by Kisch and Henry in 1956 mark the first milestone on the road of natriuretic peptide research. Using electron microscopy (EM) on guinea pig hearts, Kisch reported the discovery of atrial-specific granules which resembled electron-dense granules observed in endocrine organs (1) – the granules contained what would later be known as ANP and, to a lesser extent; BNP. The same year, Henry et al. conducted physiological experiments demonstrating that balloon distension of the left atria resulted in an increased urine flow in dogs (2). This effect depended on intact innervation, as a blockade of the cervical vagi nerve conduction route with ice abolished the effect (2). Kisch and Henry independently pointed to the localization of receptors within the atrium as being sensitive to wall stretch (1, 2). These two studies provided the first experimental evidence supporting an endocrine role of the heart in fluid volume regulation. In the 1960s and '70s, the work of Jamieson and Palade (3) extended Kisch’s findings by documenting atrial electron dense granules as being identical to neuronal and endocrine poly peptide secreting cells. In 1976, Marie et al. (4) reported that the density of these atrial granules was affected by experiments that altered fluid volume and salt load in rats. Three years later, Adolfo de Bold found that atrial-specific granules changed in response to alterations in the water-electrolyte balance (5); the number of atrial-specific granules decreased in response to water deprivation and increased by salt loading, suggesting that atrial-specific granules contain a biologically active substance involved in fluid volume regulation (5).

De Bold et al. provided the first direct evidence for a cardiac factor directly involved in fluid regulation in 1981: In a groundbreaking experiment (6), de Bold and colleagues documented a rapid and potent natriuretic and diuretic response (i.e. a reduction in systemic sodium and water retention) to intravenous (i.v.) injection of atrial extracts in rats. The extracts also had vasorelaxant activity on smooth muscle preparations (6) – thus, de Bold and colleagues linked Kisch’s and Henry’s studies by showing that atrial, but not ventricular, extracts contained a potent blood pressure reducing component that works by stimulating renal sodium and water secretion and induce vasorelaxation (6). This generated intense research activity that culminated in de Bold et al. isolating a new 28 amino acid (aa) peptide (ANP-28) from rat atrial tissue in 1983 (7).
In 1984, ANP was isolated by Matsuo and Kangawa from human atrial tissues (8). Like i.v. injection of atrial extracts had previously shown, the newly isolated peptide exhibited diuretic, natriuretic, and vasodilating effects and was labeled “cardionatrin I” (7) – more commonly known as Atrial natriuretic peptide (ANP) today.

In 1983 and 1984, shortly after the publication of de Bolds’ paper, a number of groups reported the purification and sequencing of atrial peptides of varying sizes that possessed natriuretic, diuretic and/or smooth muscle cell relaxing activity (9-13). The atrial peptides were given several different names; atrial natriuretic factor, atriopeptin, cardiodilatin and others, but atrial natriuretic peptide (ANP) is the term most frequently used to describe this peptide in the current literature. Following the discovery of ANP, Sudoh and colleagues isolated a peptide they labeled brain natriuretic peptide from porcine brain in 1988 (14). However, the cardiac ventricles were subsequently found to be the major source of circulating BNP (15) and B-type natriuretic peptide (BNP) is the name commonly used today.

In 1990, Sudoh et al. isolated C-type natriuretic peptide (CNP) from porcine brain (16). CNP is widely expressed in the central nervous system (and other tissues), but the vascular endothelium is the major site of CNP synthesis (17). In 1992, Dendroaspis natriuretic peptide (DNP) was identified in the venom of the green mamba (Dendroaspis angusticeps) (18). Some studies indicate that DNP is also present in humans (19). However, sufficient evidence supporting DNP expression in human tissues has not been presented and no gene expressing DNP has been identified in humans. Some literature also lists other members of NPS, such as Urodilatin (URO), first described in 1988 (20), long-acting natriuretic peptide (LANP), kaliuretic peptide, vessel dilator and others. However, with the exception of URO and DNP, these peptides will not be discussed further.

Throughout the 1980’s and 1990’s the biological effects, kinetics and therapeutic potential of the NPs were thoroughly investigated. The NPs were shown to be secreted into the systemic circulation in response to myocardial stretch (21) and that they inhibit the RAAS and SNS (sympathetic outflow), promote natriuresis, diuresis and vasodilation in arteries, veins and resistance vessels (arterioles), increase stroke volume and other biological effects (12, 21-28). The effects of synthetic versions of ANP (Anaritide, Carperitide) and BNP (Nesiritide) were also investigated in small populations of heart failure (HF) patients. Carperitide and Nesiritide were later approved for treatment of acute (decompensated) heart failure (ADHF) in Japan in 1995 and the USA in 2001, respectively (13). The history of these therapeutic agents will be discussed in more detail in the results and discussion sections.
To summarize, an experiment conducted by Henry and colleagues in 1956 demonstrating increased urine flow in dogs after balloon distention of the atria (2) and Kisch’s discovery of atrial granules resembling secretory (endocrine) cells in guinea pigs the same year (1), marks the beginning of natriuretic peptide research. Another milestone in the history of natriuretic peptide research was the ground breaking (1981) experiment by Adolfo De Bold and colleagues which successfully demonstrated that intravenous injection of atrial extracts had potent natriuretic, diuretic and vasorelaxant effects in rats (6) – by linking Kisch’s and Henry’s previous findings and the results of the 1981 experiment, de Bold and his colleagues showed that atrial extracts contained a potent blood pressure reducing component that works by stimulating renal sodium and water secretion. It might be more accurate to say that the natriuretic peptide research field emerged from this discovery as this lead to intense research activity that culminated in the discovery of the first natriuretic peptide (ANP) – first in rat atria by de Bold in 1983 (7) and one year later also in human atria by Matsuo and Kangawa (8). Five years after the discovery of ANP, Sudoh et al. isolated another natriuretic peptide from porcine brain extracts (14) called brain- or B-type natriuretic peptide (BNP). Two years later, Sudoh et al. isolated a third natriuretic peptide, C-type natriuretic peptide (CNP), also from porcine brain extracts (16). The biological and physiological properties of the natriuretic peptides were thoroughly investigated throughout the 1980’s and 1990’s. The natriuretic peptides were shown to be released in response to myocardial stretch and hypertensive and hypervolemic states and, once released to the systemic circulation, were able to induce several blood pressure lowering, renal and cardioprotective effects. The biological effects of the natriuretic peptides easily led researchers to the idea of therapeutic use of these agents (in both cardiovascular and renal disease states). Shortly after the discovery of the natriuretic peptides, synthetic versions of ANP (Anaritide, Carperitide) and BNP (Nesiritide) were developed and tested in small patient populations. Carperitide and Nesiritide were approved for treatment of ADHF in Japan in 1995 and the USA in 2001, respectively. Synthetic (recombinant) forms of natriuretic peptides, such as Anaritide, Carperitide, Nesiritide and Ularitide, will be discussed in the results section.
1.2 The basics of natriuretic peptides

The natriuretic peptides (NPs) are a group of genetically distinct, but structurally and functionally related polypeptides that play an important role in cardiovascular, renal and endocrine homeostasis. The structural similarity between natriuretic peptides lies in a 17 amino acid (aa) ring structure linked by a disulfide bond between two cysteine residues (fig. 1 in the appendix). The NPs are also functionally related in that they elicit similar biological effects, such as promoting diuresis (increased urine volume), natriuresis (excretion of sodium) and vasodilation, which cumulatively reduces blood volume and blood pressure (BP), inhibition of cardiac and vascular remodeling through both their hemodynamic and endocrine actions and a diverse array of other biological effects (13).

Most literature on the natriuretic peptide system (NPS) lists atrial-, B-type and C-type natriuretic peptide (ANP, BNP and CNP) as its three principal members. Others include a group of NPs often referred to as “the ANP family”, consisting of ANP (aka ANP-28; a peptide consisting of 28 aa residues), atrial natriuretic factor IV (ANF IV; ANP-25), urodilatin (URO, ANP-32), long-acting natriuretic peptide (LANP), vessel dilator and kaliuretic peptide. The peptides of “the ANP family” are produced by alternative processing of the ANP prohormone (proANP) and elicit biological effects typical of natriuretic peptides (natriuresis, diuresis, vasodilation, etc.). With the exception of ANP-28 and URO, however, these peptides lack the 17 aa ring structure which is typical, and perhaps a defining trait, of the NPs. Consequently, some would argue that these peptides are not “rightful” members of the NP family. The intestinal peptides guanylin and uroguanylin are also listed as members of the NPS in some of the literature and some also include Dendroaspis natriuretic peptide (DNP), a NP originally isolated from the venom of the green mamba (18), in the NPS in humans. The presence of the latter in humans, however, is the subject of some controversy (29-31) and no gene encoding DNP has yet been reported. Other members of the NPS in humans may exist, but this will not be discussed any further. The reason for listing all these members of the NPS in humans, legitimate or not, is to provide a frame of reference as synthetic (recombinant) forms of ANP (Anaritide; ANP-25, Carperitide; ANP-28), BNP (Nesiritide), URO (ANP-32; Ularitide) and various “designer peptides”, such as Cenderitide (CD-NP), a chimeric peptide containing CNP fused together with the elongated C-terminal tail sequence of DNP, have been developed as therapeutic agents and will be discussed in more detail in the results section.
1.2.1 Structure, synthesis, plasma half-life and clearance of NPs

The structural similarity of the NPs lies in a 17 aa ring structure linked by a disulfide bond between two cysteine residues (13). In other words, the different NPs have different size and primary structures, but they all contain a 17 aa ring within their structure (fig. 1). The NPs are the products of three separate genes; natriuretic peptide precursor A (NPPA) and NPPB, located on chromosome 1, and NPPC, located on chromosome 2. Transcription of these genes results in the formation of three different prepropeptides of approximately 130 aa called preproANP (13, 32), preproBNP (13) and preproCNP (13, 33). The prepropeptides are enzymatically cleaved to form the propeptides proANP, proBNP and proCNP after removal of a signal peptide sequence of 25, 26 and 23 aa, respectively (13). The propeptides are further processed to form mature and biologically active natriuretic peptides (ANP-28, BNP1-32, BNP4-32, CNP-53, CNP-22 and URO) and biologically inactive N-terminal propeptide segments called NT-proANP, NT-proBNP, NT-proCNP (13). The main enzymes involved in this process are Corin and Furin (13) (fig 2).

The propeptide proANP (126 aa) contain four peptide hormones within its structure, numbered by their aa sequences beginning at the N-terminal end of proANP, called long-acting natriuretic peptide (LANP; aa 1 to 30), vessel dilator (aa 31 to 67), kaliuretic peptide (aa 79 to 98) and ANP (aa 99 to 126; ANP-28 is also referred to as ANP-99-126 for this reason) (34). LANP, vessel dilator and kaliuretic peptide circulate as distinct peptides after being proteolytically cleaved (35, 36) and mediate biological effects typical of NPs (37-39), but lack the 17 aa ring structure typical of NPs.

URO (ANP-95-126) is primarily produced in the kidneys by alternative processing of proANP (20, 40) and, unlike the other members of “the ANP family” (except ANP-28), contain 17 aa ring within its structure (typical of NPs) (20).

Transcription of NPPB produces preproBNP (134 aa), which is further processed to proBNP (108 aa); proBNP(1-108) is cleaved upon secretion to produce the biologically active BNP1-32 or BNP4-32 and a 76 aa biologically inactive segment known as NT-proBNP (41). The BNP prohormone is also present in the circulation and processed to mature, biologically active BNP (BNP1-32) (42). Transcription of NPPC produces preproCNP (126 aa), which is converted to proCNP (103 aa) and enzymatically cleaved to form CNP-53 (53 aa) and CNP-22 (33, 43). These peptides (CNP-53 and CNP-22) are equipotent (44, 45) and have similar activity and functions, but CNP-53 predominates in the heart, central nervous system (CNS) and endothelium, whereas CNP-22 predominates in the cerebral spinal fluid and plasma (46).
The NPs are synthesized in the heart, vasculature, central nervous system (CNS), the lungs, kidneys, bone and other tissues, and in various cell types, such as cardiomyocytes, fibroblasts, endothelial cells, etc. (13). The NPs are differently expressed throughout the body, but their main sites of synthesis are the heart (ANP, BNP), the vasculature, CNS (CNP) and the kidneys (URO) (13, 40). The NPs are secreted from these tissues in response to various stimuli. For instance, ANP and BNP are released in response to stretching of the atrial and ventricular myocardium (in states of increased intravascular volume and/or blood pressure), pressor hormones, tissue hypoxia, cold and other stimuli (47). Secretion of CNP is mainly stimulated by growth factors (48, 49), shear stress (50) and profibrotic mediators, such as transforming growth factor-β (TGF-β) (51).

The plasma half-life of ANP is approximately 2-5 minutes in normal human subjects (52, 53). CNP has the shortest plasma half-life of only 2-3 minutes (54), whereas BNP has a half-life of approximately 20 minutes (55, 56). The plasma levels of ANP are relatively low (10 fmol/ml) in healthy subjects, but elevated from 10- to 30-fold in patients with congestive heart failure (CHF) (27, 57). The BNP plasma concentration is approximately 2 pmol/ml in healthy human subjects (58), but dramatically elevated in HF patients (55, 56). The mean concentration of CNP is very low (only 1 fmol/ml) in normal subjects, but also elevated in CHF (59-61).

The NPs are cleared from the circulation by binding to natriuretic peptide receptor C (NPR-C), which leads to receptor-mediated internalization and lysosomal degradation (62). Some studies also suggest that the NPs are eliminated by glomerular filtration (63, 64). Another clearance pathway of the NPs is through enzymatic degradation by neprilysin (NEP; neutral endopeptidase 24.11) (65), insulin degrading enzyme (IDE) (66) and Dipeptidyl peptidase-IV (DPP4) (67) (see fig 3. in the appendix). Other enzymes, such as Endothelin converting enzyme (ECE), may also be involved in the degradation of NPs (68). Although several enzymes are involved in the cleavage and catabolism of NPs, therapeutic agents inhibiting NP degradation have been mainly directed at NEP (see results). For this reason, NEP will be described in more detail; NEP is primarily expressed in the renal proximal tubes, but also in myocardial and endothelial cells, leukocytes, the lungs and CNS (46). NEP hydrolyses peptide bonds at the amino side of hydrophobic residues, thereby opening their 17-residue ring structure and inactivating the NPs (65). NEP is not specific for NPs, but also involved in the degradation of vasoactive peptides (bradykinin, endothelin-1, adrenomedullin, angiotensin I), neuropeptides (substance P, opioids), β-amyloid protein, calcitonin gene-related peptide, gastrin and vasoactive intestinal polypeptide (69-72).
1.2.2 Natriuretic peptides, their receptors and signal pathways

The NPs signal in an autocrine, paracrine and endocrine manner and exert their effects by binding to three principal receptors; natriuretic peptide receptor A (NPR-A), NPR-B and NPR-C (73-79). ANP, BNP, URO and DNP preferentially binds NPR-A, whereas CNP is the sole endogenous ligand for NPR-B (80-82). NPR-C binds all NPs with high affinity (13). The NPRs are membrane-bound receptors primarily expressed in the heart, vasculature, kidneys, CNS, lungs, adrenal glands, pancreas and adipose tissue, but NPRs are also present in other tissues and various cell types (fibroblasts, leukocytes, platelet and others) (83-87). The NPRs consist of an extracellular ligand-binding domain, a transmembrane region and an intracellular domain with similar topology for NPR-A and NPR-B. The intracellular domains of NPR-A and NPR-B differs from the smaller (37 aa) intracellular region of NPR-C (13). NPR-A and NPR-B, also known as particulate guanylyl cyclase A and B (pGC-A, pGC-B), are membrane-bound homodimers that contain intracellular catalytic domains with guanylyl cyclase (GC) activity. Upon activation (ligand-binding to the receptors), NPR-A and NPR-B catalyze the conversion of guanosine triphosphate (GTP) to the second messenger 3’5-cyclic guanosine monophosphate (cGMP) in target cells (78, 79). Increased intracellular levels of cGMP activate cGMP-dependent protein kinase (aka protein kinase G; PKG), the main effector molecule of the NP signal pathways. PKG mediates most of the biological effects of NPs through protein phosphorylation and regulation of ion channels, nuclear translocation and gene expression (88-93). cGMP is deactivated by intracellular phosphodiesterases (PDEs) (94) that terminate the NP signals. NPR-C lacks the intracellular GC-domains found in NPR-A and NPR-B and the primary role of this receptor is believed to be clearance of circulating NPs through receptor mediated internalization and lysosomal degradation (13, 95). However, NPR-C is also believed to mediate some of the biological effects of NPs (especially CNP, guanylin and uroguanylin) through G protein-dependent inhibition of adenylate cyclase (AC)-activity and through phospholipase C (PLC)-interaction that produces various non-cGMP mediated biological effects (73, 96-98). The signal- and clearance pathways of the NPs are illustrated in fig. 3 in the appendix.
1.2.3 Biological effects of the natriuretic peptides

The NPs play an important role in cardiovascular, renal and endocrine homeostasis and mediate a diverse array of biological effects both within and outside these systems. The NPs are important regulators of fluid and electrolyte balance (6, 7, 24, 25, 27), serve a counter-regulatory role to the renin-angiotensin-aldosterone system (RAAS) (24, 25, 99-101) and the sympathetic nervous system (SNS) (102, 103), induce vasodilation in both arteries, veins and resistance vessels (arterioles) (6, 23, 24, 27, 54) and help preserve the structural integrity of the heart and blood vessels through inhibition of cardiac and vascular remodeling, that is; hypertrophy (pathological growth of cardiomyocytes) and fibrosis (formation of excess fibrous connective tissue due to fibroblast proliferation, myofibroblast transformation, extracellular matrix production; collagen synthesis, etc.) (104-111). However, the biological effects of the NPs are not limited to the cardiovascular, renal and endocrine systems – the NPs also exert several metabolic effects (112) and CNP is a well-known participant in longitudinal bone growth (endochondral ossification) (113-115). Several studies also suggest that the NPs are involved in neuronal development (116), neuroprotection (117) and reproduction (118). A detailed description of the biological effects of the NPs will not be provided, but the major physiological actions of the NPs are listed in table 1 in the appendix.
1.3 Natriuretic peptides and heart failure

Although much focus has been on the therapeutic role of natriuretic peptides (NPs), their role as biomarkers represents a far more successful and perhaps most useful approach to the use of NPs in the clinical setting. As previously mentioned, ANP and BNP are released from the atria and ventricles to the systemic circulation in response to myocardial stretching and pressor hormones (epinephrine, angiotensin, vasopressin, etc.) (119). In other words; the plasma levels of these NPs and their N-terminal segments increase in hypertensive and hypervolemic states and in states of cardiac stress, such as heart failure (HF). Thus, measurements of the plasma levels of NPs and their N-terminal segments are reflective of disease states and can be used in the clinical setting. Numerous investigations into NPs as diagnostic biomarkers have been made and NT-proBNP and BNP are apparently most useful. Measurements of NT-proBNP is recommended as a biomarker for diagnosis in acute and chronic symptomatic HF and for prognosis at all stages of HF (120, 121). Meta-analyses of controlled trials also suggest titration of therapy in chronic HF according to serial measurements of NT-proBNP, as an adjunct to guideline-mandated management, improves outcomes (121, 122). Measurements of NPs and N-terminal segments are also often used in clinical trials as surrogate outcomes reflecting the effect of treatment and severity of disease. The NPs also play an important role in the pathophysiology of CHF (discussed in a later section) and many, if not most, of the studies investigating the therapeutic role of NPs have focused on NPs as potential novel therapeutics in the treatment of HF. Despite considerable therapeutic advances in recent years, HF remains a severe medical condition and socio-economic burden and there is still a compelling need for novel therapeutics. In this section, we will present general aspects on HF and review the role of NPs in the pathophysiology of HF and explain why the NPs are considered to hold great therapeutic potential in HF therapy.

1.3.1 Definitions of heart failure

Heart failure (HF) can be defined as a complex clinical syndrome caused by structural and/or functional cardiac abnormality resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress (120). The syndrome is characterized by dyspnea, edema and fatigue (breathlessness, ankle swelling and a subjective feeling of tiredness) that may be accompanied by signs caused by elevated jugular venous pressure, pulmonary crackles and peripheral oedema (120).
1.3.2 Classification

There are several ways to classify HF; acute- (decompensated; ADHF), chronic-, left-, right-, HF with reduced ejection fraction (HFrEF, aka ‘systolic HF’), heart failure with mid-range ejection fraction (HFmrEF), HF with preserved ejection fraction (HFpEF, aka ‘diastolic HF’) and congestive HF (CHF) (120). According to the New York Heart Associations (NYHA) functional classification (123), HF can also be classified in four categories or stages (I-IV) (1) depending on the patients exercise capacity and symptomatic status (present during rest and severity of HF symptoms) (120, 123). The American College of Cardiology Foundation-American Heart Association (ACCF/AHA) stages of HF (124) also provide useful and complementary information about the presence and severity of HF based on structural changes and symptoms. The ACCF/AHA stages of HF emphasize the development and progression of disease and can be used to describe individuals and populations, whereas the NYHA classes focus on exercise capacity and the symptomatic status of the disease (120).

1.3.3 Aetiology of heart failure

HF can be caused by several medical conditions and pathological states including diseased myocardium (ischemic heart disease, toxic damage, immune-mediated and inflammatory damage and infiltration; malignancy, amyloidosis, sarcoidosis, haemochromatosis, etc.), metabolic derangements (diabetes, thyroid- and parathyroid diseases, growth hormone deficiency, metabolic syndrome, etc.), genetic abnormalities, abnormal loading conditions (hypertension, valve and myocardium structural defects, pericardial and endomyocardial pathologies; constrictive pericarditis, pericardial effusion, etc. and high output states; severe anemia, sepsis, etc.), volume overload (renal failure or iatrogenic fluid overload) and arrhythmias (tachy- and bradyarrhythmias in the atria or ventricles) (120). Many patients will have several different pathologies, cardiovascular and non-cardiovascular, that conspire to cause HF (120).
1.3.4 The natriuretic peptide system in the pathophysiology of heart failure

The pathophysiology of heart failure (HF) is complex and there are several different mechanisms and pathological processes (hypertrophy, fibrosis, neuroendocrine activation, sodium and water retention, etc.) involved in the development and progression of HF (125). Which of the (many possible) mechanisms and pathological processes are most important/prominent depends in part on the etiology (hypertension, valvular defects, ischemic heart disease, etc.), co-morbidity (concomitant diseases, such as diabetes, metabolic syndrome, renal failure, etc.), genetics and other factors (120, 125). It is also necessary to specify the “subtype” of HF (acute, chronic, congestive, HF with reduced or preserved ejection fraction, etc.), considering that the pathophysiology of heart failure with reduced ejection fraction (HFrEF, systolic HF) is much better understood and differs greatly from the pathophysiology of heart failure with preserved ejection fraction (HFpEF; diastolic HF) - this is also reflected in that there are several effective treatment options for HFrEF patients (ACE-I; angiotensin converting enzyme inhibitors; Enalapril, ARBs; angiotensin receptor blockers; Valsartan, β-blockers; Metoprolol, etc.) that improve survival and reduce morbidity (120), but no effective treatment options that reduce mortality and morbidity in HFpEF patients are available at the time of writing (120) - with the possible exception of mineralocorticoid receptor antagonists (MRA) and angiotensin receptor neprilysin inhibitors (ARNI) (126). However, their effects on mortality, morbidity and hospitalization in HFpEF patients are still unclear (but several studies investigating these potentially novel therapeutic agents in the treatment of HFpEF are scheduled to be completed later this year; see results).

Chronic heart failure is a syndrome with a complex pathophysiology characterized by an early activation of different neurohormonal systems, such as the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) in the presence of left ventricular (LV) systolic dysfunction (causing inadequate circulatory blood flow to meet the metabolic demands of vital organs, such as the brain and kidneys) (127-130). The inadequacies in blood flow resulting from systolic dysfunction¹ and decreased cardiac output (CO) also activate other regulatory systems, such as the natriuretic peptide system (NPS), kinins, endothelin, erythropoietin, prostaglandins and adrenomedullin (131-134). Activation of SNS and RAAS

¹ Systolic dysfunction = an inefficient ability of the heart muscle (ventricular myocardium) to contract
promote peripheral vasoconstriction and increases systemic vascular resistance (SVR). These compensatory mechanisms aim to restore circulatory homeostasis. However, prolonged activation of these systems (RAAS and SNS) becomes detrimental and contribute to progression of HF (cardiac remodeling; hypertrophy, fibrosis) and worsening symptoms (135). The natriuretic peptide system (NPS) exert biological effects that are counter-regulatory to the RAAS and SNS (136-138), including inhibition of cardiac remodeling and natriuretic, diuretic, vasodilative effects that reduces blood pressure (BP) and blood volume. The biological effects of NPs are considered to be long-term beneficial, whereas the RAAS and SNS responses are short-term beneficial, but detrimental in the long-term. However, in chronic HF the effects of endogenous NPs is inadequate to counteract the detrimental effects of the RAAS and SNS. There are several possible explanations for this (as discussed below). The pathophysiology of congestive heart failure (CHF) is also characterized by sodium and water retention; a decrease in cardiac output (CO) and effective intra-arterial volume lead to renal retention of sodium and water despite expansion of the extracellular fluid-volume (139). In CHF patients, sodium and water retention occurs even though there is a progressive activation of the NPS (140). However, the NP response is evidently insufficient to counteract the effects of SNS and RAAS-activation in this setting as well. A possible explanation for this could be that the SNS and RAAS suppress secretion of NPs and thus lower their plasma levels, which leads to a shift in the balance between these endocrine systems and, consequently, peripheral vasoconstriction and progressive sodium and water retention. This idea is supported by the results from a few studies (141, 142). On the other hand, other studies have consistently reported elevated plasma levels of NPs in HF patients (27, 56, 57, 60). Progression of sodium retention and vasoconstriction might also be explained by attenuated renal responsiveness to NPs (especially ANP in HF patients). If so, the attenuated response is most likely linked to down-regulation of natriuretic peptide receptors (NPR-A and NPR-B), in a state of RAAS overstimulation, rather than reduced synthesis of NPs - this claim is supported by the results from several preclinical studies (143-145). Alternatively, synthesis of dysfunctional NPs due to abnormal processing in failing hearts could be another plausible explanation; production of dysfunctional NPs with reduced bioactivity would shift the balance between the NPS and RAAS/SNS in favor of promoting RAAS and SNS effects, considering the counter-regulatory roles of these systems (136-138). This could explain why there is vasoconstriction and sodium and water retention, rather than natriuresis, diuresis and vasodilation, despite elevated plasma levels of NPs (27, 56, 57, 60).
Yet another possible explanation is a combination of the explanations we’ve discussed so far (e.g. synthesis of dysfunctional peptides and NPR down-regulation). One author believes renal hypo-responsiveness to NPs (especially ANP in HF patients) is probably multifactorial (146). This could have great therapeutic implications; if HF represents a state of deficiency (lack of fully functional mature NPs), developers of novel therapeutics should focus on exogenous agents similar to native, fully functional, NPs (e.g. peptides that are structurally similar or identical to mature NPs, or non-peptide NPR agonists or positive allosteric modulators), rather than enhancing/augmenting the endogenous NPS through inhibition of NP degradation (e.g. NEP inhibition, NPR-C antagonists, etc.). Alternatively; a novel therapeutic approach could be to augment the NPS through inhibition of NP degradation and use exogenous agents analogous to native NPs or non-peptide NPR-A and -B agonists or positive allosteric modulators.

Considering the biological actions of NPs, albeit insufficient to preserve sodium and water balance in a state of SNS and RAAS overstimulation, it is reasonable to presume that NP release in hypervolemic and hypertensive states is an important compensatory mechanism aiming to reduce peripheral vascular resistance, effective blood volume and blood pressure. The beneficial effects of pharmacological therapy targeting the RAAS and SNS (ACE-I, ARBs, β-blockers, etc.) and the counter-regulatory roles of the RAAS/SNS and the NPS provide rationale for therapeutic use of NPs (augmentation of the NPS), inhibition of RAAS/SNS, or both. NP augmentation can be achieved by the use of exogenous agents that are able to activate NPRs and/or by use of inhibitors of NP degradation (NEP-I, NPR-C antagonists, etc.).
2 Methods

Literature search
I searched PubMed using the terms natriuretic peptides, therapy, treatment and the names of various therapeutic agents\textsuperscript{2} for studies published between 1950 and 2019 investigating or reviewing the role of natriuretic peptides as therapeutic agents. I also reviewed the reference section of all included studies and relevant reference articles were reviewed in full-text. The detailed search strategy is described in the section “Search in PubMed”

Eligibility criteria
The studies included were reviews, systematic reviews, meta-analyses, comparative- or clinical studies (clinical trials, phase I-IV-trials, randomized controlled trials, etc. see “article types” in section “Search in PubMed”). The articles had to be published in peer-reviewed journals, written in English and appear relevant\textsuperscript{3} to the subject in their title or abstract

Data collection
605 articles were identified through database searching (as per January 28, 2019) and 23 articles identified through other sources. The articles from other sources were mostly recently published review articles subjectively considered to be of high quality. These were included mainly to provide “background information” (introduction section). I read all the titles and abstracts retrieved by the literature search and assessed the abstracts to make sure they met the eligibility criteria. 138 articles were selected for full text review after reviewing their titles and abstracts for eligibility. The remaining articles were excluded. Three full texts were excluded due to language barriers (English abstracts, full text in Czech, Chinese and French). After completing the full text reviews, I searched clinicaltrials.gov \textbf{(https://clinicaltrials.gov/ct2/search)} and PubMed as described in the sections “search in clinicaltrials.gov” and “Additional searches in PubMed”.

\textsuperscript{2} “Various therapeutic agents” refers to therapeutic agents I knew of before I started writing my thesis. These are listed in the search thread described in the section “Search in PubMed”

\textsuperscript{3} The articles were considered relevant if they summarized previous studies/trials on NPs as therapeutic agents (reviews, systematic reviews and meta-analyses) or investigated the therapeutic role of NPs in HF or other diseases themselves; all phases of clinical trials, selected preclinical trials, meta-analyses, etc. (see section “search in PubMed, specifications; article types”)
Search in PubMed

To search in PubMed, I used the following thread:

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I used the following specifications:

**Article types:** Clinical study, Clinical trial, Clinical trial phase I-IV, Comparative study, Controlled clinical trial, Guideline, Meta-analysis, Multicenter study, Practice Guideline, Randomized controlled trial (RCT), Review, Systematic review

**Publication dates:** From 01/01-1950 to 31/12-2018

**Language:** English

Search in clinicaltrials.gov

Based on the information gathered from the articles selected for full text review, a search in clinicaltrials.gov was conducted using the following terms: Anaritide, Carperitide, Nesiritide, Ularitide, Vasonatrin, Cenderitide, CU-NP, ANX-042, ASBNP.1, M-ANP, Mutant ANP, ZD100, BMN111, AC-NP, CNAAC, racemodotril, candoxatrilat, UK-73967, Ecadotril, Omapatrilat, Sampatrilat, Fasidotril, alatriopril, LCZ696 and Entresto. This produced a various number of results (ranging from none to 75), incl. studies on other diseases than HF.

Additional searches in PubMed

A link to the published results was often not provided in clinicaltrials.gov. Therefore, additional searches in PubMed were conducted using the name of the study (ADHERE, OVERTURE, PARADIGM, ASCEND-HF, etc.) and/or the relevant therapeutic agent (Anaritide, Cenderitide, CU-NP, etc.) with one specification; publication dates set to the year the study was scheduled to be completed plus 5 years.
3 Results

Based on the literature obtained by searching PubMed and clinicaltrials.gov, all therapeutic agents targeting the NPS augment this system by various mechanisms and can be categorized as either recombinant NPs, designer NPs or inhibitors of natriuretic peptide (NP) degradation. Recombinant NPs include recombinant forms of ANP (Anaritide, Carperitide), BNP (Nesiritide) and Urodilatin (Ularitide). These agents are structurally and functionally identical to their endogenous counterpart (structure, plasma half-life, target receptors, etc.)

Designers NPs are engineered (chimeric) peptides produced by combining different parts of the structure of native NPs or adding specific aa sequences to their structure. Most of the designer NPs developed were developed by investigators at the Mayo clinic.

Inhibitors of NP degradation can be further classified as neprilysin inhibitors (NEP-I), angiotensin converting enzyme neprilysin inhibitors (ACE/NEP-I; aka Vasopeptidase inhibitors) and angiotensin receptor neprilysin inhibitors (ARNI). A newly developed ARNI called Entresto (formerly known as LCZ696 and contain Sacubitril/Valsartan) represents the most successful use of a therapeutic agent targeting the NPS in the history of NPs as therapeutic agents. Therefore, this agent will be described in more detail.

3.1 Recombinant natriuretic peptides

Recombinant NPs are synthetic peptides with the same primary structure (amino acid sequence) as endogenous NPs (Fig 4 in the appendix). Recombinant NPs were the first therapeutic agents targeting the NPS investigated for clinical use, that is; in the treatment of various cardiovascular and renal diseases, especially HF. Based on my search results, four agents that can be placed in this category have been developed; recombinant forms of ANP (Anaritide, Carperitide), BNP (Nesiritide) and URO (Ularitide). In 1995, Carperitide (recombinant ANP) was approved by the Ministry of Health and Welfare of Japan for intravenous administration in patients with acute heart failure (AHF) or acutely decompensated HF, but Nesiritide (recombinant BNP) was not approved for therapeutic use in Japan (147). In 2001, Nesiritide was approved for clinical use by the Food and Drug Administration (FDA) in the USA (13, 147). For this reason, the evidence for therapeutic use of Carperitide and Nesiritide has been compiled mainly to Japan and the USA, respectively.
3.1.1 Recombinant ANP (Anaritide, Carperitide)

ANP was first discovered in 1983 (then labeled “Cardionatrin I”) (7). In the following years, numerous peptide variants of ANP were discovered, but according to Potter and colleagues “subsequent studies [have] revealed that the mature form of ANP is 28 amino acids (aa) and that smaller versions are degradation products that maintain various levels of activity” (13). Among the smaller peptides, the most widely studied is the 25-residue version of human ANP (lacking the first three residues) called atrial natriuretic factor 4 (ANF IV, ANP-25) and its synthetic form Anaritide. Anaritide has been used in extensive clinical trials investigating the cardiovascular and renal effects in healthy and non-healthy subjects.

In 1986, a study conducted by Cody et al. demonstrated that Anaritide infusions cause natriuresis, diuresis, decreased systemic blood pressure and decreased pulmonary capillary wedge pressure (PCWP) in healthy volunteers (27). However, Anaritide failed to elicit statistically significant natriuretic and diuretic effects when administered to patients with congestive heart failure (CHF) (27). The following year (1987), Saito et al observed a similar lack of diuresis and natriuresis when patients with CHF were infused with the mature form of ANP (148). In a later study involving a larger CHF patient cohort, Fifer et al found that infusion of Anaritide promote the characteristic renal effects of NPs. However, the response was less than that of the normal/healthy subjects (149). Saito et al. reported similar effects with Carperitide, the full-length (recombinant) form of human ANP, in a study completed in 1987 (148).

In Japan, clinical studies on the effectiveness of mature ANP continued and in 1995 Carperitide was approved for the treatment of acute decompensated heart failure (13). Investigations were also initiated to study the effectiveness of ANP in the treatment of renal disease. The first trial was conducted by Rahman et al in 1994 and involved 53 patients (150). The aim of the study was to evaluate the ability of Anaritide infusion to reduce the need for dialysis in patients with acute tubular necrosis. Their results suggested a positive outcome for patients receiving Anaritide because an increased creatinine clearance and a decreased need for dialysis was observed in the patients that participated in the trial (150). The results of this trial led to the initiation of a multicenter placebo-controlled clinical trial involving 504 patients with acute tubular necrosis and their results were published in 1997. The authors concluded that 24 hour-infusion of Anaritide did not improve the overall survival of the patients without dialysis, but their results indicated that a subset of patients might have benefited (151). Despite the lack of effect on mortality, a second trial involving 222 patients
with oliguric acute renal failure (renal failure resulting in urine production less than 400 ml per day) was conducted by Lewis and colleagues and their results were published in 2000. Their results indicated no statistically significant benefit of Anaritide in dialysis-free survival (152). Both trials remarked on the severe hypotension often following Anaritide-infusions. Indeed, it is this severe hypotension that appears to be limiting the utility of Anaritide as a therapeutic agent for either heart failure or renal disease – Lewis et al. even stated in their discussion that “it is possible that if this hypotension could be avoided, anaritide would have been efficacious” (152).

Investigations on the ability of Anaritide to prevent radiocontrast-induced nephropathy – a common cause of acute renal failure (an increase in blood urea nitrogen or serum creatinine above a certain level within 24 hours) occurring within 48 hours after exposure to intravascular radiographic material (153) – were also initiated. However, the results from a clinical trial involving 247 patients conducted by Kurnik and colleagues in 1998 demonstrated that Anaritide along with hydration was no more effective at preventing radiocontrast-induced nephropathy than hydration alone (154).

In 2004, studies conducted in Sweden compared the ability of furosemide, a loop diuretic, and mature ANP (28 aa) to increase GFR (glomerular filtration rate), renal blood flow and reduce renal oxygen consumption in patients with acute renal failure. The authors concluded that furosemide was a more effective agent (155).

Despite the potent natriuretic and diuretic effects of synthetic (recombinant) ANP analogs in healthy subjects, clinical studies conducted to date indicate little or no therapeutic benefit of ANP analogs in the treatment of renal disease. The underlying mechanisms responsible for the lack of efficacy of NPs in the treatment of renal disease are unknown, but a possible explanation may be downregulation of NPR-A in the renal medulla and/or upregulation of NPR-C in the renal cortex (156).

As previously mentioned, Carperitide was approved for the treatment of acute decompensated heart failure (ADHF) in Japan in 1995. Several studies has been conducted since then. In 2005, Suwa and colleagues reported that Carperitide infusion clinically improved 82 % of (acute) heart failure patients, with the efficacy of the drug greater in patients with decompensated chronic heart failure (157). In 2008, the results from the COMPASS study (Carperitide Effects Observed Through Monitoring Dyspnea in Acute Decompensated Heart Failure Study) were published (158). The COMPASS study was a prospective observational study involving 1832 patients with ADHF. The patients received Carperitide as monotherapy
and 83.2% recovered from the acute phase according to their results. The results from the PROTECT multicenter randomized controlled study were published the same year (159). Their results suggested that low-dose Carperitide infusions improved the long-term prognosis of ADHF patients (159). However, this was a small study involving only 49 patients and larger studies are needed to determine the therapeutic value of Carperitide in terms of prognosis. The results from more recent and larger studies published in 2015 and 2017 suggest that Carperitide infusions is associated with increased in-hospital mortality rate in ADHF patients (160) or has no significant effect on mortality (161). The results from two studies published in 2017 also indicate that Carperitide is not a cost-effective therapeutic option and also associated with negative outcomes in HF patients (162, 163).

Opinions regarding the use of ANP analogs in the treatment of acute heart failure and kidney disease are varied (164). Although synthetic (recombinant) ANP analogs have been shown to successfully restore some hemodynamic parameters following HF and clinical improvement in patients with renal disease, whether ANP analogs ultimately reduce mortality and their long-term effects are unknown (165). Recent studies indicate no effect or increased risk on mortality (160, 161) in HF patients and larger studies need to be conducted to determine the safety and efficacy of Carperitide.
3.1.2 Recombinant BNP (Nesiritide)

BNP was discovered in 1988 (14). At the time of discovery, BNP was assumed to elicit responses (biological effects) similar to those of ANP (natriuresis, diuresis, vasodilatation, etc.) due to the structural similarity between these two peptides. BNP was almost immediately viewed as a potential candidate for treating both cardiovascular and renal diseases. In 1992, four years after the discovery of BNP, Nesiritide was patented in the U.S.A. Nesiritide is a purified preparation of human BNP manufactured from Escherichia coli using recombinant DNA technology. Nesiritide has the same 32 aa sequence as the endogenous BNP (BNP1-32) produced in the ventricular myocardium (166) – In other words; Nesiritide is a synthetic peptide structurally identical to endogenous BNP (BNP-32). Nesiritide may be administered by intravenous bolus, intravenous infusion or subcutaneous injection (24, 167-169). The onset of action following an intravenous bolus injection occurs within 15 minutes and the effects lasts for at least 4 hours (170). The hemodynamic effects following intravenous infusion occurs after approximately 45 minutes (170). Nesiritide has a half-life of approximately 18 minutes (170) (slightly shorter than that of BNP, which is about 22 minutes) and elicits the same biological effects as endogenous BNP.

The first report on the biological effects of Nesiritide in healthy subjects and patients with congestive heart failure came from Yoshimura and colleagues in 1991. The year before, McGregor et al. had demonstrated that administration of porcine BNP resulted in a natriuretic response, increased urinary excretion of cGMP and reduced plasma aldosterone concentrations (25). The following year, Yoshimura et al. reported that infusion of Nesiritide (the recombinant form of human BNP) resulted in diuresis, natriuresis, increased secretion of chloride, decreased pulmonary capillary wedge pressure (PCWP) and systemic vascular resistance (SVR), increased stroke volume, increased plasma-ANP and decreased plasma aldosterone concentrations (24). Unlike ANP, infusions of BNP/Nesiritide elicited these effects not only in healthy volunteers, but also in patients with congestive heart failure (24). Throughout the 1990s several studies were conducted to investigate the cardiovascular, endocrine and renal effects of BNP/Nesiritide in healthy subjects and patients with congestive heart failure (and other diseases) (169, 171-179). Some suggested a possible role for BNP and other NPs in the treatment and diagnostics of renal and cardiovascular diseases; especially heart failure, but also in other pathological states such as pulmonary- and essential hypertension, (post) myocardial infarction and other diseases (167-169, 171-179). These studies, however, were conducted on relatively small patient populations, often involving only
10 subjects or less. The results from these early studies were very promising, but of limited value considering the small number of test subjects. The authors acknowledged the limitations of their results and addressed the need for larger studies to be conducted to determine the effects and clinical usefulness of BNP/Nesiritide.

In 1999, Mills et al. examined the effectiveness of 24 hour-infusion of Neseritide to 103 patients with congestive heart failure in a randomized, double-blind, placebo-controlled multicenter trial (180). Their results demonstrated that Neseritide reduced both preload and afterload, resulting in an increase in stroke volume and cardiac output. However, this study presented no data on mortality or symptomatic effects (except a few cases of hypotension which were mostly asymptomatic) (180).

The following year, the results from an efficacy trial and a comparative trial conducted by the Nesiritide Study Group (Colucci et al) (181) were published. In the efficacy trial, 127 patients were randomly assigned to receive Nesiritide (0.015 or 0.03 μg/kg/min) or placebo for six hours to determine the effect of Neseritide on hemodynamic parameters (pulmonary-capillary wedge pressure; PCWP), global clinical status, dyspnea and fatigue (181). In the comparative trial, 305 patients were randomly assigned to open-label therapy with standard agents or Neseritide for up to seven days. The results from the efficacy trial indicated that Neseritide-infusions reduced PCWP, improved the global clinical status and reduced dyspnea and fatigue significantly compared to placebo (181). In the comparative trial, improvements in global clinical status, dyspnea and fatigue were sustained with Neseritide therapy for up to seven days with a similar effect to that of standard intravenous therapy for heart failure (181). The most common side effect was dose-related hypotension, which was usually asymptomatic.

The results of these trials (180, 181) likely led to the approval of Neseritide, marketed under the trade name Natrecor, for the treatment of acute decompensated heart failure (ADHF) by the US Food and Drug Administration (FDA) in 2001 – after initial non-approval (13). At the time of approval, the effects of Neseritide infusions and bolus injections had been investigated in “more than 700 [HF] patients” (170), but the results from the studies conducted prior to FDA’s approval of Natrecor (Neseritide) had only demonstrated hemodynamic improvement, improved clinical status and reduced dyspnea (167, 169, 170, 180, 181) – no data regarding the effect of Neseritide on mortality and morbidity had been presented when Neseritide was approved for the treatment of ADHF. The initial “New Drug Application” was first filed with the FDA in 1998 (182). One year later, before their approval, the FDA did request more data due to the limited amount of evidence on the safety and efficacy of Neseritide-use in ADHF
patients. Initially, the FDA did not approve Nesiritide for use in ADHF therapy. Following FDA’s request, a study involving just over 400 patients was completed- and their results published in 2000 (181). However, this study only demonstrated symptomatic relief and improved hemodynamic parameters in ADHF patients receiving Nesiritide (adverse events included dose-related hypotension) – these results are not particularly earth-shattering and present no data on the effect of Nesiritide on mortality and morbidity. No results from large studies on Nesiritide were conducted between 2000 (when these results were published) and 2001 (when FDA approved Nesiritide for use in ADHF therapy). Therefore, one must assume that the FDA regarded these results as satisfactory evidence and sufficient proof to launch a new drug – Despite the absence of data on important clinical outcomes, such as mortality and morbidity.

In 2002, a larger multicenter trial called the Vasodilation in the Management of Acute Congestive Heart Failure (VMAC)-study (183) was completed and their results published. The VMAC-study compared the effects of the addition of nitroglycerin or Nesiritide versus placebo to standard therapy in 489 patients with dyspnea at rest from decompensated congestive heart failure. The group treated with Nesiritide had improved dyspnea after three hours of treatment compared to placebo, but there was no significant difference in dyspnea or clinical status with nesiritide compared to nitroglycerin. After 24 hours of treatment, the reduction in PCWP was marginally greater in the Nesiritide group than in the nitroglycerin group (-8.2 mmHg and -6.3 mmHg, respectively) and the patients reported no significant differences in dyspnea and only modest improvement in global clinical status (183). Furthermore, the VMAC study found that nesiritide infusion produced fewer adverse cardiovascular effects than nitroglycerin (183). The results from the PRECEDENT study (Comparative and Prospective Randomized Evaluation of Cardiac Ectopy With Dobutamine or Nesiritide Therapy) were published the same year (184). The PRECEDENT study (184) compared the effect of Nesiritide (0.015 or 0.03 μg/kg/min) and dobutamine (≥ 5 μg/kg/min), a commonly used therapeutic agent in the treatment of ADHF, on ventricular arrhythmias in the treatment of 255 patients with ADHF. Their results indicated that Nesiritide was a safer short-term treatment option in ADHF-therapy compared to dobutamine due to the frequent occurrence of arrhythmias and tachycardia associated with dobutamine use (184). A study published the following year concluded that “nesiritide appears cost effective relative to dobutamine as treatment for patients with acutely decompensated HF” (185). Despite the lack of sufficient data on important outcomes such as mortality, length of stay, readmission rates
and adverse effects besides headache and hypotension (especially renal function), hopes were initially high for Nesiritide – the first new drug approved for heart failure therapy in 14 years (166, 170, 186-188).

In 2003, Nesiritide became commercially available in Israel and Switzerland under the trade name Noratak (13). Except for Switzerland, Nesiritide was not approved in Europe and approval was delayed pending further investigations into the renal responses of Nesiritide infusion (13).

In 2004, Wang et al. reported that Nesiritide did not improve renal function in patients with chronic heart failure (189) and, the following year (2005), Sackner-Bernstein and colleagues published two meta-analyses of Nesiritide indicating that it worsened renal function and increased the risk of death (190, 191). In one of the meta-analyses, Nesiritide use in 862 patients during ADHF hospitalization was associated with a 74% increased risk of 30-day mortality ($p = 0.059$) (191). In the other, Nesiritide use in 862 patients during ADHF hospitalization was associated with a 54% increased risk of worsening renal function (190).

Several subsequent studies, including Acutely Decompensated Heart Failure Registry (ADHERE) (192), have disputed this claim (192-198). The results from the BNP-CARDS study (NCT00186329) published in 2007 also suggested that Nesiritide had no detrimental effect on renal function when cohorts of similar baseline renal function were compared (195). However, this was only a 75-person study so larger studies were needed to determine if Nesiritide really impaired renal function and increased the mortality rate.

In 2008, the results from FUSION II (Safety and efficacy of outpatient nesiritide in patients with advanced heart failure: results of the Second Follow-Up Serial Infusions of Nesiritide; NCT00091520), a trial involving a total of 911 patients, were published (193). FUSION II (193) was a randomized, double blind, placebo controlled trial that examined the safety and efficacy of serial outpatient Nesiritide administration to heart failure patients. The trial found no evidence of worsening renal function or increased mortality. However, conclusions from this trial also state that “Serial outpatient nesiritide infusions do not provide a demonstrable clinical benefit over intensive outpatient management of patients with advanced American College of Cardiology/American Heart Association stage C/D heart failure.” (193). Due to the conflicting results and continued concerns on the safety and efficacy of nesiritide, the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF; NCT00475852) clinical trial was designed. ASCEND-HF (199) is the largest study conducted on Nesiritide to date and involved 7141 patients that were randomly assigned to receive either
Nesiritide or placebo in addition to standard care for 24 to 168 hours (199). The coprimary end points (outcomes) were the change in dyspnea at 6 and 24 hours and the composite end point of rehospitalization for heart failure or death within 30 days. The results from this study were published in the New England Journal of Medicine in 2011. According to their results, nesiritide had no impact on the rate of death and rehospitalization, nor was it associated with worsening renal function (199). The authors also stated that nesiritide only had a small, nonsignificant effect on dyspnea when it was used in combination with other therapies and that neseritide-use was associated with an increase in rates of hypotension. Nesiritide improved symptoms in the European, but not in the United States patient cohort with ADHF and was not superior to conventional therapy in improving mortality in patients with ADHF in both continents (199). In their conclusions, the authors stated that “On the basis of these results, nesiritide cannot be recommended for routine use in the broad population of patients with acute heart failure” (199).

To summarize, Nesiritide is a recombinant form of human BNP that has been shown to improve hemodynamic parameters (decreased PCWP, etc.) and the global clinical status and reduce dyspnea and fatigue compared to placebo (181). However, based on the results of the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF), the largest study on Nesiritide conducted to date, Nesiritide has no effect on mortality in patients with ADHF and is associated with symptomatic hypotension and other adverse events. Nesiritide was approved for the treatment of ADHF in the USA in 2001 (13). Two subsequent reports (published in 2005 by Sacker-Bernstein and colleagues) of an increased risk of worsening renal function and death compared with control therapy raised doubts on its safety (190, 191). Consequently, a trial involving over 7000 patients labelled ASCEND-HF was initiated. This study demonstrated that nesiritide had no impact on the rate of death, nor was it associated with worsening renal function (199), as previously claimed by Sackner-Bernstein and colleagues. However, nesiritide was associated with increased rates of hypotension. The authors’ statement read as follows; “On the basis of these results, nesiritide cannot be recommended for routine use in the broad population of patients with acute heart failure” (199). In February 2018, the FDA reported that Jansen Pharmaceuticals was discontinuing the manufacture of Nesiritide (200).
3.1.3 Recombinant urodilatin (Ularitide)

Ularitide is the synthetic form of urodilatin (URO; ANP-32, ANP95-126). The endogenous form of URO is a 32 aa NP structurally similar to ANP (ANP-28) that plays a pivotal role in the physiologic regulation of fluid-balance and sodium homeostasis (40). URO is believed to be produced in the kidneys through local synthesis and/or differential processing of renal or circulating pro-ANP (the same precursor as ANP-28 produced in the atria) (20, 201, 202). The mature form of the peptide is secreted into the urine and exists in low levels naturally in the systemic circulation (20). URO stimulates cGMP production by binding to NPR-A (pGC-A), which leads to increased urinary flow, glomerular filtration rate (GFR), sodium excretion and urinary cGMP excretion (20, 203, 204).

URO was discovered in 1988 (20) and its biological properties and physiological actions were determined in preclinical studies and phase I clinical trials published a few years after its discovery (201, 203, 204). The results from these studies suggested a potential role in the treatment of cardiovascular and renal disease states, including heart failure (HF), and so several studies investigating the therapeutic potential, and possible role, of Ularitide in the treatment of cardiovascular and renal disease states were conducted. In an initial open-label study conducted by Kentsch and colleagues (published in 1992) (205), Ularitide was administered to 18 healthy volunteers as intravenous (IV) bolus injections at doses of 1, 2, or 4 µg/kg 15 minutes after placebo (0.9% saline). The pulmonary capillary wedge pressure (PCWP) started to decrease 5 minutes after Ularitide was administered and remained below baseline until 90 minutes post-administration in a dose-dependent manner (205). This effect was paralleled by increased plasma levels and urinary excretion of cGMP. Additionally, hemodynamic effects following an IV bolus injection of 2 µg/kg ANP were considerably less pronounced compared to all doses of Ularitide (205). The results from this study indicated that Urodilatin might exhibit beneficial effects in cardiovascular disease. Shortly after, the same group conducted a randomized, open-label study involving 12 NYHA class IV acute heart failure (AHF) patients (206). This study compared the effects of Ularitide vs. ANP, administered as IV bolus injections at a dose of 4 µg/kg, on various hemodynamic parameters, such as PCWP and systemic vascular pressure (SVR), cardiac index and stroke volume, as well as urine flow and sodium excretion. Ularitide was shown to have stronger hemodynamic effects compared to ANP and decreased PCWP to a greater extent in these patients (206). These results merited further investigations into the therapeutic potential of Ularitide.

In one of the first randomized, placebo-controlled, double-blind studies in humans, published
in 1995, twelve patients with stable HF (NYHA functional class II or III) received Ularitide or placebo (0.9 % saline) (207). The aim was to determine the efficacy of a prolonged infusion of urodilatin (15 ng/kg/min for 10 hours) on various hemodynamic, renal and endocrine parameters; systolic blood pressure, mean arterial pressure and central venous pressure decreased and the heart remained unchanged during Ularitide infusion, whereas only minimal changes in these parameters was seen in the placebo group (207). There was no statistically significant difference in urine flow. However, this might simply be due to high variability in the Ularitide group and the small number of patients included in the study. Furthermore, sodium excretion was increased in Ularitide group and unchanged in the placebo group. The neurohormonal levels (plasma concentrations of aldosterone, norepinephrine, vasopressin and renin) did not change during Ularitide or placebo infusion (207). In their conclusion, the authors stated that “Prolonged infusion of urodilatin lowers preload and increases diuresis and natriuresis without neurohumoral activation or adverse side effects, demonstrating a profile of effects that may be beneficial in patients with CHF [congestive heart failure]”(207). Although this study did not demonstrate a statistically significant increase in diuresis in response to Ularitide infusions compared to placebo (as claimed by the authors), the remaining results from this study indicate that URO has a role in the treatment of CHF.

The results from the phase I clinical trials led to the initiation of larger studies (phase IIa and IIb clinical trials), most notably the SIRIUS (Safety and efficacy of an Intravenous placebo-controlled Randomized Infusion of Ularitide in a prospective double-blind Study in patients with symptomatic, decompensated chronic heart failure) studies; SIRIUS I (IIa) and SIRIUS II (IIb). These trials represent the phase II program that would further explore the potential clinical value of Ularitide in acute decompensated heart failure (ADHF).

SIRIUS I (208) was a randomized, double-blind, ascending dose-safety study of 24 patients with ADHF (NYHA functional class III or IV) requiring hospitalization and monitoring via right heart catheterization, cardiac index (CI) ≤ 2.5 L/min/m2 and PCWP ≥ 18 mmHg). The aim of the study was to determine the efficacy of 24-hour intravenous infusions of urodilatin (at doses of 7.5, 15 or 30 ng/kg) or placebo in the treatment of ADHF. The results were published in 2005; compared to baseline, urodilatin decreased PCWP, right atrial pressure and NT-proBNP levels, improved dyspnea and the plasma levels of cGMP were significantly higher in the intervention (Ularitide) group compared to the control (placebo) group (208). Overall, there was no obvious difference in the safety profiles of the different groups. However, there were three cases of transient asymptomatic hypotension (systemic BP < 90
mmHg) in the groups receiving Ularitide at doses of 15 or 30 ng/kg (208).

One year later (2006), the results from SIRIUS II (209) were published. SIRIUS II was a (phase IIb) randomized, double-blind, placebo-controlled study that included 221 patients with ADHF (matching the criteria of SIRIUS I) receiving either placebo or Ularitide at doses of 7.5, 15 or 30 ng/kg/min as 24 hour continuous infusion, in addition to standard therapy. The aim of SIRIUS II was to determine the efficacy and safety of Ularitide in treating patients with ADHF using the same hemodynamic parameters as in previous studies (PCWP, SVR, CI, etc.), the patients’ self-assessed changes in dyspnea using a 7-point Likert scale (prior to hemodynamic assessments) and various renal parameters (serum levels of creatinine, urea, etc.) as outcomes. Ularitide demonstrated a significant decrease in PCWP, improved dyspnea, reduced SVR and increased CI compared to placebo. Heart rate and serum creatinine levels were unchanged in all groups (209). The most frequent adverse event was dose-dependent hypotension which occurred in a number of patients. The authors concluded that Ularitide lowered cardiac filling pressures and improved dyspnea without apparent deleterious effects on renal function (209) - suggesting that Ularitide may play a role in HF treatment. The effects of Ularitide on renal function were investigated in more detail (13) using the following outcomes; (the effect of Ularitide on) estimated glomerular filtration rate (GFR), serum creatinine, creatinine clearance and blood urea nitrogen (BUN). The authors concluded that overall, renal function was not impaired by Ularitide throughout the 24 hour infusion and during a 2-day follow-up period (210).

To summarize, in preclinical studies (animal models) of HF, Phase I and II clinical trials, Ularitide demonstrated beneficial effects on hemodynamic parameters and HF symptoms (dyspnea), while still preserving renal function. However, these studies were conducted on small patient populations and larger studies needed to be conducted.

The beneficial clinical effects and promising surrogate results led to the initiation of a randomized, double-blind, placebo-controlled Phase III study in patients hospitalized with an episode of acute HF called TRUE-HF (Efficacy and Safety of Ularitide for the Treatment of Acute Decompensated Heart Failure; NCT01661634) in 2012 (211, 212). The aim of this study was to evaluate the effects of Ularitide infusion on the clinical status and cardiovascular mortality of ADHF patients compared to placebo. TRUE-HF included 2157 patients with AHF that were randomly assigned to receive continuous intravenous infusion of either Ularitide, at a dose of 15 ng/kg/min, or placebo for 48 hours in addition to standard therapy. The coprimary outcomes were death from cardiovascular causes during a median follow-up of
15 months and a hierarchical composite end point that evaluated the initial 48-hour clinical course (i.e. a composite score of assessing ‘worsening heart failure’ that included a patient global assessment compared with baseline using a 7-point Likert scale of symptoms, persistent or worsening HF and all-cause mortality at 6, 24, and 48 h after start of study drug infusion). The study was completed in 2016 and the results were published in 2017; Ularitide exerted favorable physiological effects (e.g. greater reductions in systolic blood pressure compared to placebo) and reduced the levels of NT-proBNP, but did not affect cardiac troponin levels (211). Furthermore, short-term treatment did not affect a clinical composite end point or reduce long-term cardiovascular mortality (211). Despite encouraging results in preclinical studies and Phase I-IIb clinical trials, the results from TRUE-AHF demonstrated a lack of efficacy on important clinical outcomes. A meta-analysis that was recently published (December 2018) concluded that Ularitide has demonstrated high effect sizes for pulmonary artery wedge pressure (PAWP) and right atrial pressure and improvements in these parameters were greater with ularitide vs. pooled data for other vasoactive drugs (213). The authors also provide several possible reasons for why the favorable hemodynamic effects obtained in “proof of concept” Phase II-studies, as well as improvements in other surrogate markers, such as NTP-proBNP, did not result in a significant decrease of mortality in Phase III-trials; “These [reasons] include short duration of infusion time (24–48 h), fixed treatment dose regimen, protocol violations and the failure to use guideline-directed therapy in the majority of Phase 2 trials. The hypothesis underlying the use of vasoactive substances in ADHF—that rapid reversal of ventricular wall stress preserves myocardial viability—may itself be plausible, but cannot be translated into a mortality benefit. Another possibility is that the reduction of micro-myocardial injury by unloading the ventricle is insufficient to influence long-term outcomes” (213).

Although a potential role in the treatment of HF and other diseases using Ularitide or other recombinant agents cannot be completely ruled out (despite disappointing results from large Phase III-trials), the available data on Ularitide indicate a story erringly similar to that of Nesiritide.
3.2 Designer natriuretic peptides

Severe hypotension, short half-life, reductions in renal perfusion pressures and the potential for reflex-induced sympathetic responses limit the clinical effectiveness of recombinant agents. In an effort to overcome these shortcomings, the development of designer NPs has emerged as an innovative advancement in drug discovery. Designer NPs (aka chimeric or hybrid peptides) are novel peptides that have been engineered through modifications in their aa structures or through use of genetically altered forms of native (endogenous) NPs (Fig. 5). The biochemical design of designer NPs can be a de novo creation or based on selected sequences of native NPs, the result of modification and/or addition of aa sequences or genetic modification of the native NPs (214).

Peptide engineering is a growing field in drug discovery and several new peptides have been developed (214). These peptides are superior to native NPs in terms of molecular mechanisms of action, specificity and stability (215) and also less susceptible to enzymatic degradation compared to native NPs (216). The designer NPs mediate the same biological effects, although to a greater extent, and/or with prolonged duration compared to native NPs. Some of the designer NPs are also able to activate both NPR-A and NPR-B, which may optimize activation of the NPS and thus indicate a greater therapeutic potential.

Although advances in engineered designer peptides have occurred more widely outside of CV disease, especially in the treatment of cancer, HIV and diabetes, this approach may represent a future direction in the treatment of CV diseases as well. Engineering of designer NPs also invoke the possibility to produce peptides with unique properties applicable to a specific syndrome (thus “personalizing” the peptide; e.g. peptides that limit hypotension and/or remodeling in HF, augment adipocyte activation in obese patients or produce other desirable “specialized” effects). The chemical properties of designer NPs can easily be changed and this contribute to their overall applicability. Synthetic peptides are also used for other purposes, such as production of antibodies, polypeptide structure and functional studies and also development of new vaccines, drugs and enzymes (214). Altogether, these novel chimeric peptides play an important role in advancing the panoply of means available for treatment of CV and renal diseases, especially HF and hypertension.
Vasonatrin
Vasonatrin (VNP) is a 27 aa hybrid NP (chimeric peptide) that was developed in 1993 by investigators working in the Cardiorenal Research Laboratory of the Mayo clinic (217). VNP represents the first attempt at a designer NP (217). VNP is a chimeric peptide consisting of the full-length 22 aa structure of human CNP (CNP-22) fused to the 5 aa carboxyl terminus (C-terminal) of human ANP (217). This structural adaptation enables VNP to retain both the venodilating properties of CNP and the ability of ANP to promote natriuresis in both in vitro and in vivo models (214). VNP also possesses arterial vasodilating and antiproliferative effects that are unique for this first-in-class chimeric peptide that defined the concept of designer NPs (214). The vasorelaxant effects of VNP are dose-dependent, endothelium-independent and stronger than the effects of ANP and CNP alone (218-220). In healthy rats, however, the natriuretic and diuretic effects of a single bolus injection of 50 μg/kg VNP is inferior to ANP (217). This observation led to reduced enthusiasm and the clinical development of VNP was terminated shortly after (214).

Cenderitide
Cenderitide (CD-NP), a designer NP invented by Dr. Burnett and colleagues in 2008 and later patented by the Mayo Clinic (214), is a 37 aa hybrid NP (chimeric peptide) which represents a first-in-class designer NP able to co-target both NPR-A and NPR-B. Cenderitide is designed by fusing CNP-22, which possesses venodilating properties, but limited renal actions and minimal effects on blood pressure, with the 15 aa carboxyl terminus (C-terminal) of DNP (Fig. 6) (214). The C-terminus of DNP itself has less hypotensive actions than DNP, but still possesses natriuretic properties (214). Endogenous CNP (CNP-22) primarily binds to NPR-B and does not promote natriuresis and diuresis (at least not to the same extent as ANP and BNP). However, CD-NP retains the vasodilative, antifibrotic and antihypertrophic effects of CNP/activation of NPR-B, as well as the natriuretic and diuretic actions of DNP/activation of NPR-A (221). In other words; CD-NP can activate both NPR-A and NPR-B and is thus able to mediate biological effects of several NPs. The drug design strategy for Cenderitide was, indeed, based on two objectives; engineering a peptide which could co-activate both NPR-A (like ANP, BNP, URO and DNP) and NPR-B (like CNP), under the assumption that this would optimize activation of the NPS. This strategy was also believed to avoid excessive hypotension after treatment - as were the case following treatment with Nesiritide/NPR-A activation; as demonstrated in the ASCEND-HF and Renal Optimization Strategies Evaluation in Acute Heart Failure (ROSE-HF) trials (222).
CD-NP has been shown to elicit potent natriuretic and diuretic responses, increased glomerular filtration rate (GFR), inhibition of renin, reduced atrial and systolic blood pressure, inhibition of cardiac fibrosis and mediate several cardiac-unloading effects (216, 221, 223-225). CD-NP is also less likely to induce hypotension compared to Nesiritide (139). Several clinical trials designed to assess the safety and tolerability of IV administration of CD-NP and the dose relationship of CD-NP on improvement of clinical symptoms and renal function in HF patients have been conducted since CD-NP was first developed. In 2011, the FDA provided a fast-track designation for CD-NP in Phase II clinical trials for chronic therapy in patients with post-acute HF (224). A Phase I-trial aiming to determine the safety and efficacy of chronic subcutaneous administration of CD-NP in HF patients with left ventricular assist device (LVAD) support - a mechanical device designed to improve hemodynamic function - is currently ongoing and scheduled to be completed in 2020 (NCT03091998). Although the therapeutic role of CD-NP in this setting is still unknown, a study completed in 2014 indicate that CD-NP has therapeutic potential in the treatment of end-stage HF patients receiving LVAD support (226).

The most recent completed study involving CD-NP is a phase I-trial called BELIEVE III (“CD-NP [Cenderitide] Therapy for the Preservation of Left Ventricular Function Post Anterior Myocardial Infarction - Pilot Study”; NCT02071602). This trial was completed in November 2018 and was a "proof of concept", randomized, double-blind, placebo controlled study, involving 60 patients admitted with a first time ST elevation anterior STEMI (myocardial infarction of the anterior cardiac wall). However, the results from this study have not yet been published.

Although several studies investigating the safety and efficacy of CD-NP in HF patients have been completed, most of which were conducted between 2007 and 2018, no results demonstrating an effect on important clinical outcomes, such as a reduction in mortality or morbidity in response to CD-NP treatment, have yet been published. In fact, the results from several trials involving CD-NP has not been published at all and some of the studies were withdrawn according to clinicaltrials.gov. In other words; the future role of CD-NP in the treatment of HF (and other cardiorenal disease states) might not be as prominent as initially assumed (227) despite much optimism and a demonstrable therapeutic potential (based on the reported biological effects); the observed effects in previous animal and (brief) human studies involving 20-100 subjects indicate that CD-NP without a doubt has therapeutic potential in
the treatment of cardiorenal diseases and may represent a new cardiorenal therapeutic, but the ability of CD-NP to reduce mortality and morbidity, improve clinical symptoms or elicit beneficial effects on other important clinical outcomes, has not been demonstrated. It’s also worth noting that the results from a study investigating the effects of CD-NP on murine adipose tissues in vitro and in vivo, published in 2017 (228), indicate that CD-NP treated mice exhibit a significant increase in body mass, worsened glucose tolerance and hepatic steatosis in response to CD-NP treatment. The same study concluded that long-term CD-NP treatment results in an increased expression of NPR-C (“the natriuretic peptide scavenger receptor”) and decreased lipolytic activity. In their conclusion, the authors stated that “NP effects differed depending on the duration of treatment […] raising questions about the rational of natriuretic peptide treatment in obese patients” (228). On the other hand, the effect of CD-NP on hard endpoints that actually matter (i.e. reduced mortality and morbidity, symptomatic relief, etc.) are not available at the time of writing. On the other hand, the lack of results on these outcomes does not necessarily rule out a therapeutic role for CD-NP and, in its defense, the results from the first-in-human study was published only a few years ago. In this study (227), the investigators demonstrated that CD-NP possess cyclic guanosine monophosphate (cGMP)-activating, natriuretic and aldosterone-suppressing properties in normal human volunteers (227), indicating a possible role as a new cardiovascular therapeutic. In addition to these results, several animal studies and human trials have demonstrated that CD-NP is safe and improves cardiovascular and renal function without inducing significant levels of hypotension. Early data also suggest improved renal function in heart failure patients in response to CD-NP treatment (229).

According to Burnett and colleagues, the first clinical target for Cenderitide was HFrEF in the post acute HF period (222), but HFpEF is also considered to be a condition in which the NPS represent a potential therapeutic target due to cGMP-activating, RAAS-inhibiting, antiproliferative and antifibrotic effects of CD-NP and the importance of these elements in the pathophysiology of HF (222). Other disease states in which CD-NP might have a therapeutic role is pathological states characterized by reduced cardiac function associated with chronic kidney disease (CKD) and fibrosis. Various types of HF (ADHF, CHF, etc.) might also represent suitable arenas for CD-NP as a therapeutic agent. One important question in this context, however, is whether the effects of CD-NP observed in preclinical studies and in healthy volunteers can be translated to the state of HF. The results from the first prospective, randomized, placebo-controlled trial to determine the overall safety and tolerability of
Cenderitide, as well as the ability of Cenderitide to activate plasma and urinary cGMP in patients with stable chronic HF, were published in 2018 (230). This study tested the hypothesis that CD-NP can be safely administered to HF patients and that the activation of cGMP observed in preclinical studies and healthy volunteers could be translated to HF. In this study, 18 stable HF patients participated and 12 of them were randomized to receive CD-NP, whereas the remaining 6 patients received placebo. The major findings of this study were that a 4 hour infusion of CD-NP was safe, well tolerated with increases in plasma cGMP and urinary cGMP excretion without inducing hypotension. An important observation was that GFR was preserved and increased in response to a GFR reduction. There were no adverse effects, including (no) changes in blood pressure (230). These results support further investigations of Cerneteside in HF as a potential future cGMP-enhancing therapeutic strategy. The effect of CD-NP on important clinical outcomes such as mortality and morbidity, however, is still unknown and despite its therapeutic potential, a definitive conclusion on whether CD-NP represents a novel cardiorenal therapeutic agent cannot be made based on the available results. That being said, CD-NP does seem to have a remarkable antifibrotic effect. This is important since nearly all etiologies of heart disease involve pathological myocardial remodeling characterized by excessive deposition of extracellular matrix (ECM) proteins by cardiac fibroblasts, which leads to reduced tissue compliance and accelerated progression towards HF, as well as poor prognosis in HF patients. Thus, the antifibrotic effects of CD-NP may represent a significant advance in the treatment of cardiovascular disease states characterized by cardiac fibrosis, and its potential to suppress cardiac fibrosis in both HFrEF and HFpEF is a credible hypothesis. However, until an effect on important clinical outcomes has been demonstrated, CD-NP remains a therapeutic agent with great potential, but cannot yet be regarded as a new therapeutic agent suitable for the treatment of cardiorenal diseases based on the available results. In conclusion, Cenderitide was a major advance in peptide engineering and now has proven efficacy in humans (at least to some extent); two Phase II studies of 8-day treatment with continuous subcutaneous administration by Insulet OmniPod delivery system in stable HF patients have been completed at the time of writing and several ongoing studies investigating the therapeutic role of CD-NP are scheduled to be completed within the next year. However, data on mortality and morbidity, has not yet been presented. Several preclinical and clinical trials have established a therapeutic potential for CD-NP and it is reasonable to assume that the biological effects of this designer NP represent a significant advance in NP design.
CU-NP
CU-NP, a humanized version of Cenderitide, was developed by the Mayo clinic following the encouraging results in both experimental and human studies on Cenderitide (231). CU-NP was designed to preserve the favorable actions of urodilatin (URO) while minimizing the chances of hypotensive effects (231). The solution was to fuse the 17 aa ring structure of native CNP with both the C- and N-termini of URO, essentially replacing the ring structure of URO with that of CNP, to produce a novel designer NP. Although CU-NP is in the early stages of drug development, initial experimental studies have demonstrated that IV infusion of CU-NP activates cGMP in a canine HF model (232). CU-NP has also been shown to exert cardiac-unloading and renal-enhancing actions without inducing excessive hypotension, inhibit RAAS and mediate antihypertrophic effects through inhibition of the sodium-hydrogen exchanger (NHE-1)/calcineurin pathway (231, 233). Other biological effects of CU-NP include increased plasma levels and urinary excretion of cGMP, enhanced GFR and diuretic and natriuretic effects (234).

ANX-042
ANX-042 (ASBNP.1) is a renal-specific peptide based on genomic medicine also developed by investigators at the Mayo clinic. This 42 aa designer NP is produced by fusing a 16 aa segment from the C-terminal of ASBNP, an alternative spliced transcript of BNP with a unique 34 aa C-terminal, fused to the remaining (26 aa) structure of native BNP (235). The alternative spliced transcript for BNP results from intronic retention, is present in failing human hearts and is reduced following mechanical unloading. The intron-retained transcript would generate a unique 34 aa C-terminal while maintaining the remaining structure of native BNP (235). Despite its relationship to BNP, ANX-042 is not able to stimulate cGMP production in vascular smooth muscle cells or endothelial cells and, unlike BNP, does not induce vasodilation or reduce systemic (mean arterial) blood pressure according to the results from initial investigations (214). However, subsequent studies revealed that ANX-042 is able to induce cGMP production in renal mesangial cells and isolated glomeruli - surprisingly to a larger extent than BNP (although this might simply be explained by the unexpected ability of this compound to activate NPR-B or possibly other, yet unidentified, NPR (pGC) subtypes that may be present in the kidneys, but not in the vasculature) (214). ANX-042 has been shown to inhibit RAAS, increase GFR and promote natriuresis and diuresis (235). However, it lacks the ability to stimulate cGMP production in vascular cells and promote vasodilation despite its relation to BNP. Consistent with its distinct in vivo
effects, the activity of ANX-042 may not be explained through binding and activation of NPR-A and possibly not by activation of NPR-B either (235).

In 2012, ANX-042 was approved by the FDA as an investigational new drug and has now begun a first-in-human clinical trial as a designer renal-enhancing and non-hypotensive NP and this could make ANX-042 a novel renal-selective agent for treatment of HF (231). Furthermore, data from a Healthy Volunteer Dose Escalation Study (NCT01638104) showed that infusion with ANX-042 is safe and also that it results in cGMP-production. ANX-042 is currently being tested as a potential novel non-hypotensive and renal-enhancing treatment for HF. “Safety and Efficacy of ANX-042 in Human Cardiorenal Syndrome” (ANX-042 Aim 1; NCT03019653), the most recent study on ANX-042, started in 2017 and is scheduled to be completed in July 2023.

**Mutant ANP**

Mutant ANP (M-ANP, ZD100, mANP), a peptide resembling ANP, is the newest designer NP developed by investigators at the Mayo clinic (214). M-ANP was initially identified as a “mutant” peptide resulting from a mutation in the ANP-encoding gene (NPPA). This mutation results in the formation of a 40 aa peptide with a 12 aa C-terminal extension instead of the 28 aa peptide (ANP-28) with a 5 aa C-terminal extension that is normally produced after transcription of this gene (236). The newly identified peptide was initially labeled “Mutant ANP” (236), but has also been referred to as M-atrial natriuretic peptide. Its physiological actions were investigated shortly after its discovery; the researchers soon discovered that M-ANP had significantly greater plasma cGMP activation, diuretic, natriuretic, GFR-enhancing, RAAS-inhibiting, cardiac unloading and blood pressure reducing properties compared to native ANP (236). It has now been established that M-ANP exerts greater and more sustained natriuretic, diuretic, GFR- and renal blood flow enhancing actions than ANP-28 (236). However, the underlying mechanisms responsible for this are not easily explained and conflicting results and interpretations have been presented (236, 237).

As a designer NP, M-ANP is produced by fusing ANP-28 with a 12 aa extension to its C-terminal, resulting in the formation of the 40 aa peptide described earlier in this section (238). The novel C-terminal extension renders M-ANP highly resistant to enzymatic degradation by NEP (237). This implies that the C-terminal extension represents an alternative to a nonspecific NEP inhibiting (NEP-I) strategy (216). Preliminary data from ongoing studies also indicate that M-ANP has lower affinity to NPR-C - thus enabling M-ANP to avoid important clearance pathways and increases its bioavailability - this might explain the greater
and more sustained biological effects of M-ANP in vivo compared to native ANP. M-ANP has been shown to activate NPR-A (30) and some studies suggest that M-ANP causes enhanced activation of NPR-A in response to ligand-binding to the receptor (214). However, other studies refute this claim based on results from NMR spectroscopy and state that the greater in vivo activity of M-ANP is more likely to be secondary to reduced degradation by NEP rather than enhanced activation of NPR-A (237). Although there are some conflicting results and interpretations presented in the literature, most studies investigating M-ANP have basically confirmed the results of other investigators or presented similar results. For instance, the structure, origin, biological properties, etc. of M-ANP has been thoroughly investigated, are well understood and hardly controversial. For instance, virtually all of the studies I have come across agree that M-ANP is a BP-reducing agent. However, the underlying molecular mechanisms (and various other factors) that determine this effect is not always easily explained, leaving the floor open to different, sometimes not compatible, explanations. Here’s an example: In a study using an animal model of hypertension, the investigators reported that M-ANP reduces the systemic blood pressure - these results were in accordance with previous results reported by other investigators. However, this study showed that M-ANP reduce the systemic BP via multiple mechanisms, including diuresis, vasodilation and inhibition of aldosterone. Even with its notable BP-reducing actions, M-ANP was able to enhance both GFR and renal blood flow (238). The pluripotent BP-reducing actions and concomitant enhancement of renal function indicate that M-ANP has a notable therapeutic potential and possibly a future role as an antihypertensive agent with highly attractive properties, considering the fact that systemic hypertension is commonly associated with renal dysfunction and that there is an unmet need for renal-enhancing antihypertensive agents necessary for successful treatment of hypertension. On account of its BP-reducing actions and concurrent renal-enhancing effects, M-ANP may represent a novel and/or favorable therapeutic option in the treatment of resistant HTN, acute hypertension and HF with concomitant hypertension (238). Effective treatment options for resistant HTN is still lacking despite numerous attempts at developing novel therapeutics suitable for clinical use (239). M-ANP recently completed a first-in-human study in stable HTN and in resistant-like subjects with HTN (214) and is currently in clinical development for treatment of resistant HTN. Patients with resistant hypertension do not respond to current BP-lowering agents. Consequently, this group of patients have a markedly increased risk of adverse cardiovascular outcomes (i.e. increased risk of developing cardiovascular and renal diseases), such as HF,
stroke, myocardial infarction (MI) and end-stage kidney disease (238). In other words; there is still a need for novel therapeutics and M-ANP might represent an effective treatment option for these patients. The results from a study using a canine model of acute HF in the setting of HTN, indicate that M-ANP is more effective than nitroglycerine in promoting sodium excretion, preserving GFR, unloading the heart and suppression of aldosterone (239). Actually, it is more accurate to say that the results demonstrated that M-ANP had a beneficial effect on several renal parameters, whereas nitroglycerine had no significant effects on the same parameters (239). These results support the claim that M-ANP might represent an effective therapeutic option for the treatment of HTN, acute hypertension and HF with concomitant HTN (where renal protection is a key therapeutic goal). Hopefully the results from ongoing trials investigating the safety and efficacy of M-ANP will provide sufficient evidence that favor the use of M-ANP in the treatment of resistant HTN and satisfy the needs of the many patients suffering from a condition that lacks effective treatment options despite numerous attempts to develop novel therapeutics.

M-ANP recently entered a double-blind, placebo-controlled phase I clinical trial evaluating the cardiovascular and metabolic properties of M-ANP in subjects with hypertension (HTN) and metabolic syndrome according to RS5068 genotypes (MANP-HTN-MS; NCT03781739). The MANP-HTN-MS study started in 2017 and is scheduled to be completed at the end of June 2019. Two other studies on M-ANP are currently ongoing and scheduled to be completed in December 2019 and March 2021 according to clinicaltrials.gov. The available evidence on the clinical effectiveness of M-ANP is rather limited. In fact, only one Phase I study of 3-day once daily subcutaneous (SQ) injection of subjects with resistant-like hypertension has been completed at the time of writing. So for the next 1-2 years, the role of M-ANP as a therapeutic agent will remain unclear, but the initial results of ongoing studies are promising (240).

Additional studies are underway to develop novel strategies for long-term delivery of M-ANP in a suitable formulation (i.e. a formulation that results in sustained delivery) (214). One way to achieve sustained delivery and prolonged activation of NPRs (and subsequent cGMP production induced by M-ANP) is to link the designer NP to fatty acids (an approach similar to that of antidiabetic drugs, such as glucagon-like peptide 1 analogs) (214). Preliminary studies have demonstrated the feasibility of sustained release formulation of M-ANP (ZD100) and preclinical studies are underway in experimental HTN (214). Data from a first in human Phase I trial, in which single ascending doses of M-ANP (ZD100) were administered to HTN
patients followed by SQ administration (injections) once per day for 3 days, was recently published. These results demonstrate that treatment with M-ANP (ZD100) is safe, well-tolerated and associated with the same biological effects reported in previous studies, including cGMP generation, natriuresis, enhanced GFR and suppression of aldosterone (241). Recent studies also suggest that advancements in peptide engineering may lead to the development of next generation M-ANP (ZD100) analogs, with gain of function in the activation of NPR-A (242), which may potentially provide greater clinical efficacy in the treatment of human disease as new and improved therapeutic agents are developed.

**Other designer NPs**

Apart from the designer NPs we’ve discussed so far, this group of therapeutic agents also include other members; not all designer NPs have been developed in the Mayo clinic. There are probably several other designer NPs apart from those we have discussed. In fact, when I reviewed the articles selected from the results of the search in PubMed I came across other designer NPs called BMN111, AC-NP, CNAAC and Ghrelin. Apart from the latter, these three designer NPs will be briefly discussed in the section below.

**BMN111**

BMN111 (Vasoritide) is a 39 aa peptide produced by adding 17 amino acids to the mature 22 aa (full length) form of native CNP (CNP-22). BMN111 has been described as a designer NP, a recombinant form of CNP and a CNP analog - which of these categories are most accurate is debatable, but this will not be discussed further. A search in PubMed for BMN111 produce few results and none of the articles I read provided a satisfactory description of how BMN111 is produced, its structure, design and pharmacological properties. BMN111 mediate the same biological effects as native CNP and interact with the same receptors (NPR-B and NPR-C, but not NPR-A), but BMN111 has a longer plasma half-life and increased resistance to (enzymatic) NEP degradation compared to native CNP (243). Therapeutic use of this agent seems to be limited to treatment of achondroplasia (the most common form of dwarfism) (clinicaltrials.gov). BMN111 has been shown to improve skeletal parameters in experimental animal models and some describe it as one of the most promising therapeutic agents in the treatment of achondroplasia (244). A phase 2 clinical trial evaluating the safety, tolerability and efficacy of BMN111 in pediatric patients with achondroplasia (NCT02055157) was launched in 2014 and initially scheduled to be completed in October 2017. However, it seems that the trial was extended by 18 months and only the initial results have been made available.
**AC-NP**

AC-NP is a novel 28 aa chimeric peptide produced by combining the 17 aa ring structure of CNP with the 6 aa N- and 5 aa C-termini of ANP (245). It’s worth noting that AC-NP is a designer NP not developed by investigators at the Mayo clinic. To the best of my knowledge, only two studies investigating the basic properties of AC-NP has been conducted (a search in PubMed using the term “AC-NP” produces three results and only one of the results references the NPS). I accidentally came across a second study on AC-NP. One study investigated the biological effects of AC-NP in rats and compared the physiological responses of ANP, CNP, VNP and AC-NP. The results of the first study was not exactly earth-shattering; the authors list several biological effects typical of NPs (which I won’t describe in detail) that were also mediated by AC-NP, and the only results worth noting in the second study is that AC-NP seems to be able to activate both NPR-A and NPR-B (246). However, none of these studies involved human subjects. Consequently, the therapeutic role of AC-NP will remain unclear.

**CNAAC**

CNAAC is a chimeric peptide containing the C-terminus and ring structure of ANP fused with the N-terminus of CNP. Like AC-NP, this peptide was not developed by investigators at the Mayo clinic. I conducted a quick search in PubMed and found two studies on CNAAC (from 2015 and 2017) (247, 248). The first study investigated the biological effects of CNAAC in rats and compared the physiological responses to native NPs with those of VNP and CNAAC. The results of the first study were not accessible in full text, but based on what the authors state in their abstract and reference in the second study (available in full text), the first study demonstrated that intravenous administration of CNAAC exhibit potent vasodilatory, diuretic and hypotensive effects, promote diuresis, reduce mean arterial blood pressure and increase the plasma levels and urinary excretion of cGMP in rats (247, 248). These biological effects are typical of NPs and not particularly exciting. The second study examined the effects of the chimeric peptide on left ventricular dysfunction after myocardial infarction in rats. Although the results have limited value, it’s worth mentioning that both the native NPs (ANP and CNP) and the designer NPs (VNP and CNAAC) reduced myocardial fibrosis and infarct size based on a histological analysis, but the effect was greater in designer NPs (247). Like several of the native NPs, recombinant forms, etc. CNAAC also inhibit RAAS and exerted longer duration of action compared to native NPs (247). None of these studies involved human subjects and the results that have been presented so far are few and might not be reproduced in human.
The biological effects of NPs suggest a potential role in combating cardiovascular and renal disease. However, these effects have repeatedly been demonstrated over the last 40 years and the clinical effectiveness of different forms of NPs is not proportionate to the beliefs and expectations to what such agents may accomplish. Thus, HF remains a serious condition with a high mortality rate despite development of novel therapeutics with some efficacy.

3.3 Inhibitors of natriuretic peptide degradation

Enzymatic degradation is the foremost mechanism limiting the bioavailability of NPs. Recombinant agents have structures identical to native NPs and the same basic (biological) properties, including (low) resistance to enzymatic degradation. Addition of aa or aa substitutions may produce peptides more immune to degradation with enhanced and/or prolonged biological actions. The therapeutic approaches we’ve discussed so far have focused on increasing the bioactivity of NPs through administration of exogenous agents with identical, similar or altered structures of native NPs. An alternative (possibly complementary) approach to achieve NP augmentation is to reduce the degradation of NPs. NP degradation can be attenuated by affecting NPR-C (e.g. NPR-C antagonists) or through inhibition of the enzymes responsible for NP degradation (NEP, IDE, DPP4). Numerous investigators have attempted the latter using different types of therapeutic agents that can be classified as pure NEP inhibitors (NEP-I), Vasopeptidase inhibitors or dual ACE/NEP-I (ACE; angiotensin converting enzyme, NEP; neutral endopeptidase or neprilysin) and most recently; angiotensin receptor neprilysin inhibitors (ARNI).

Pure NEP-I represent the first attempts on reducing NP degradation through NEP inhibition. However, the effects of pure NEP inhibitors were disappointing and associated with unacceptable adverse events, such as angioedema. The limitations of these agents led to the development of another group of agents, which also target NP degradation, classified as dual ACE/NEP-I. These agents inhibit both angiotensin converting enzyme (ACE) and neprilysin (NEP), but were also associated with severe adverse events (aplastic anemia). Consequently, further development of these agents was discontinued as well. The most recent group of therapeutic agents targeting NP degradation is called ARNI (angiotensin receptor neprilysin inhibitors) and LCZ696 (trade name; Entresto; Sacubitril/Valsartan), one of the ARNIs, has demonstrated a superior effect on mortality compared to Enalapril.
3.3.1 NEP inhibitors (NEP-I)

The initial attempts to inhibit NP degradation targeted neprilysin (NEP; neutral endopeptidase 24.11) using pure NEP-I, such as racedodotril (249) (oral formulation) and candoxatrilat (250) (intravenous formulation). These agents were shown to promote natriuresis, increase urinary excretion of ANP and reduce blood pressure (BP). Ecadotril, another NEP inhibitor, has been shown to elicit similar effects (251). This indicated that these agents could represent a novel therapeutic option in the treatment of hypertension and HF. However, a study on chronic use of candoxatril, the orally active prodrug of candoxatrilat (UK-73967), demonstrated that the initial reduction in blood pressure (BP) was not sustained despite continued administration. Consequently, the development of these drugs was stopped (252).

The inability of candoxatril to sustain reduced BP could be explained by the fact that NEP is not specific to NPs, but also involved in the cleavage and catabolism of several other vasoactive peptides, such as endothelin-1, adrenomedullin, angiotensin I, bradykinin, β-amyloid protein, neuropeptides (substance P, opioid), gastrin, calcitonin gene-related peptide and vasoactive intestinal polypeptide (46, 71, 72, 253). Some of these peptides are well known to elicit hypertensive effects. Thus, inhibition of this enzyme could potentially increase the plasma levels of all these substrates which could lead to conflicting hemodynamic effects and/or detrimental effects on renal and cardiac function. Moreover, NEP hydrolyzes angiotensin I to angiotensin-(1-7), which counteracts angiotensin II, and this might explain the superiority of ARNI compared to pure NEP inhibitors since ARNI drugs are able block both NEP and angiotensin receptors (AR), whereas pure NEP inhibitors have no effect on AR. (254-256).

In 1998, Cleland and Swedberg, on behalf of The International Ecadotril Multi-Centre Dose-ranging Study Investigators, concluded that ecadotril did not represent a desirable novel approach in heart failure therapy due to its lack of efficacy and that it was highly associated with aplastic anemia, a severe adverse event, in a dose-dependent manner (257). The authors stated that “…Although this study was not powered to exclude a symptomatic benefit with ecadotril, the lack even of a trend in that direction and the adverse event profile has led to cessation of clinical development of higher doses and to reassessment of the possible risk/benefit ratio for lower doses.” (257). Both candoxatril and Ecadotril are now considered to have no beneficial effects in patients with hypertension or HF (258). All in all, pure NEP inhibitors have disappointing clinical effects and this approach is now more or less abandoned.
3.3.2 Dual ACE/NEP inhibitors

Dual angiotensin-converting enzyme/neprilysin inhibitors (ACE/NEP-I, aka “Vasopeptidase inhibitors”) appeared to be the solution to the problem of pure neprilysin inhibition. The initial belief was that since NEP degrades peptides with vasodilatory and renal actions, inhibition of this enzyme ought to be beneficial in hypertension and congestive HF – especially in combination with ACE inhibitors since angiotensinogen is a substrate for ACE and that the combination of ACE/NEP-I had been shown to reduce blood pressure more effectively than either inhibitor alone (259).

Several Vasopeptidase inhibitors (dual ACE/NEP-I) have been developed, most notably Omapatrilat (BMS-186716) which had been shown to improve clinical symptoms and survival and to relieve cardiac dysfunction and hypertension in experimental HF and hypertension models (259). The therapeutic role of Omapatrilat has been evaluated in patients with hypertension or HF in the Inhibition of Metalloprotease by Omapatrilat in a Randomized Exercise and Symptoms Study of Heart Failure (IMPRESS) trial, the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE) trial and the Omapatrilat Cardiovascular Treatment versus Enalapril (OCTAVE) trial published in 2000, 2002 and 2004, respectively (260-262).

The IMPRESS trial was a prospective, randomized, double-blind, parallel trial of 573 patients with New York Heart Association (NYHA) class II-IV congestive heart failure, left-ventricular ejection fraction (LVEF) of 40% or less and receiving an ACE inhibitor. Patients were randomly assigned to receive omapatrilat at a daily target dose of 40 mg or Lisinopril at a daily target dose of 20 mg for 24 weeks. The primary endpoint was improvement in maximum exercise treadmill test (ETT) at week 12. Secondary endpoints included death and comorbid events indicative of worsening heart failure. The authors concluded that their findings suggested that omapatrilat could have some advantages in the treatment of patients with congestive heart failure (262). This led to the initiation of a larger randomized controlled trial called the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE) (261). In the OVERTURE trial, 5770 patients with New York Heart Association class II to IV HF were randomly assigned to double-blind treatment with either the ACE inhibitor enalapril (10 mg BID, n=2884) or to the ACE/NEP inhibitor omapatrilat (40 mg once daily, n=2886) for a mean of 14.5 months. The main findings of this study were that the primary end point, death from any cause or HF hospitalizations, were not reduced by omapatrilat. However, other (secondary) end points suggested a benefit with omapatrilat
(death from any cause or cardiovascular hospitalization was 9% lower in the omapatrilat group), but the rate of angioedema was much higher in the omapatrilat group (261). The relatively high rates of angioedema in the omapatrilat group can probably be explained by the fact that both ACE and NEP break down bradykinin, a compound highly associated with angioedema at increased levels, and that omapatrilat also inhibits aminopeptidase P (which is also involved in the catabolism of bradykinin). Although omapatrilat may have had some therapeutic value with regards to symptomatic relief, the lack of efficacy on reducing mortality in HF patients effectively put an end to the use of omapatrilat as a therapeutic option in HF. However, omapatrilat was still believed to have potential to be used in the treatment of hypertension. The OCTAVE trial was designed to investigate the therapeutic value of omapatrilat in hypertensive patients. This was a multicenter, randomized, double-blind, active-controlled, 24-week trial in 25,302 patients with untreated or uncontrolled hypertension conducted in 3298 office-based sites in 12 countries. Subjects were randomized to receive omapatrilat at a dose of 10 mg or enalapril at a dose of 5 mg as initial therapy for hypertension, replacement for existing antihypertensive therapy or in addition to existing antihypertensive therapy (260). The results demonstrated a mere 3.6 mmHg difference in systolic BP reduction between the omapatrilat and the enalapril group (favoring omapatrilat) after 8 weeks. As in previous trials, the OCTAVE trial also concluded that angioedema was more frequent in the omapatrilat group. Excessive potentiation of bradykinin and subsequent high rates of serious angioedema led to the discontinuation of the clinical development of omapatrilat.
3.3.3 Angiotensin receptor neprilysin inhibitors (ARNI)

Combining an angiotensin receptor blocker (ARB) and a NEP inhibitor was the logical next step and potential solution to the problems encountered with omapatrilat; an ARB was considered to be less likely to induce bradykinin potentiation, like ACE-I, and also prevent unwanted angiotensin II-induced vascular, endocrine and hemodynamic effects. Several ARNIs have been developed, but LCZ696 (trade name Entresto; Sacubitril/Valsartan) has been most successful in clinical trials and is a current therapeutic option in the treatment of selected HFrEF patients. For this reason, we will focus on LCZ696 in this section.

LCZ696 is a novel, first-in-class drug (ARNI) that was approved for treatment of HFrEF in 2015. The most recent European Society of Cardiology (ESC) guidelines on the treatment of HF assigned LCZ696 a high class of recommendation (IB) (120) and recommend it as a replacement for ACE-I (Enalapril) or ARB (Valsartan) in selected patients (120, 263). LCZ696 contains Valsartan, an angiotensin II (type I) receptor blocker (ARB) and Sacubitril (AHU377), a prodrug that is converted into the active form (LBQ657) that functions as a NEP-I. Since the active form of Sacubitril (sacubitrilat, LBQ657) does not inhibit aminopeptidase P, the risk of angioedema was expected to be lower than that of omapatrilat (264-266). LCZ696 was designed with the aim of inhibiting NEP while simultaneously blocking the adverse effects of the RAAS and reduce bradykinin potentiation (which is highly associated with angioedema, as previously discussed) (264-266). LCZ696 was also designed to be prescribed twice daily in order to guarantee an extended ARNI (24 hours). The route of administration is in the form of oral tablets at 100–400 mg/day (267) and the single dose of 200 mg delivers the equivalent of 160 mg Valsartan (266, 268).

Several studies that were recently completed and multiple ongoing clinical trials aim(ed) to determine the therapeutic role of LCZ696 in the treatment of several cardiovascular and renal diseases, such as heart failure with preserved ejection fraction (PARAGON-HF; NCT01920711, PERSPECTIVE; NCT02884206, PARALLAX; NCT03066804), diastolic dysfunction (PARABLE; NCT02682719), acute decompensated HF (TRANSITION; NCT02661217), CKD and HF (NCT03771729), chronic HF with sleep apnea syndrome (NCT02915160), essential hypertension (NCT01785472, NCT01615198, NCT01876368, NCT01193101), reducing HF after myocardial infarction (PARADISE-MI; NCT02924727) and pulmonary artery pressure reduction (PARENT; NCT02788656), as well as clinical trials that further investigate the therapeutic role of LCZ696 in the treatment of HFrEF (OUTSTEP-HF; NCT02900378, PIONEER and others).
According to clinicaltrials.gov (using the search term “LCZ696”) there are 75 studies on LCZ696/Entresto. The results from several of these trials have been published, but many of the trials were just recently completed and the results have not yet been published. Some of the studies are scheduled to begin this year, but are still in the stage of recruiting patients. A detailed description of each study will not be presented here. Instead we will focus on the trial that led to the approval of LCZ696 in the treatment of HFrEF and briefly discuss other important trials.

The results from two studies investigating the role of LCZ696 in the treatment of hypertension (HTN) are available; one comparing LCZ696 to placebo and one comparing LCZ696 to Valsartan (265, 269). Approximately 360 and 1200 patients, respectively, were included in these studies. Both of these were randomized, double-blind, placebo-controlled phase 2 clinical trials. The major findings from these trials were that LCZ696 was well tolerated with no cases of angioedema, which indicated a favorable safety profile of the drug, and that LCZ696 elicited BP reducing effects significantly better than placebo (265, 269). This indicated that LCZ696 could be beneficial in the treatment of patients with HTN and CV diseases characterized by vasoconstriction, pulmonary overload and neurohormonal activation. However, more results are needed to determine the role of LCZ696 in the treatment of HTN.

Prior to the approval of LCZ696 for treatment of HFrEF in 2015, the role of LCZ696 in HF therapy was investigated in both HFpEF and HFrEF patients in the PARAMOUNT-HF and the PARADIGM-HF trials, respectively. The results from the PARADIGM-HF trial led to the approval- and ESC guideline recommendation of LCZ696 and the results from PARAMOUNT-HF indicate that LCZ696 may have a role in the treatment of HFpEF – a condition for which there are no effective treatment options (270-272).

The PARAMOUNT-HF (Prospective comparison of ARNI with ARB on Management Of heart failUre with preserved ejectioN fraction; NCT00887588) was a phase 2, randomized, parallel-group, double-blind multicenter trial in patients with NYHA functional class II-III, LVEF 45% or higher and NT-proBNP greater than 400 pg/mL (273). The trial included approximately 300 HFpEF patients that were randomly assigned to receive 200 mg Sacubitril/Valsartan or 160 mg Valsartan twice daily for 36 weeks. The trial was completed in 2011 and the results were published in 2012. According to the results, LCZ696 reduced NT-proBNP to a greater extent than Valsartan at 12 weeks and was well tolerated (273). Despite these somewhat encouraging results, the authors stated that “Whether these effects would
translate into improved outcomes needs to be tested prospectively.” In other words, the role of LCZ696 in the treatment of HFpEF is still unclear and it remains to be seen if the reduced levels of NT-proBNP will translate into reduced mortality or other important outcomes in larger trials. The potential use of LCZ696 in the treatment of HFpEF is currently under investigation in the ongoing PARAGON-HF trial (NCT01920711).

The PARAGON-HF trial is a Phase III randomized, double-blind, parallel group, active-controlled, event-driven trial comparing the long-term efficacy and safety of valsartan and Sacubitril/Valsartan in 4822 HFpEF patients (LVEF ≥45%), NYHA functional class II to IV symptoms, elevated NP plasma levels and evidence of structural heart disease (274, 275). The primary outcome is the composite of cardiovascular death and total (first and recurrent) HF hospitalizations. The trial is estimated to be completed on May 31, 2019.

The Prospective comparison of ARNI with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF; NCT01035255) (276-278), the trial that led to the approval of Entresto in the treatment of HFrEF, was a multicenter, randomized, double-blind, parallel group, active-controlled study that evaluated the efficacy and safety of Sacubitril/Valsartan (at a dose of 97 mg/103 mg twice daily) in reducing the primary end point of cardiovascular death or HF hospitalization in 8399 HFrEF patients compared to Enalapril (at a dose of 10 mg twice daily) – one of the most commonly used angiotensin-converting enzyme inhibitors (ACE-I) in HF therapy (120, 263, 276-278). The run-in period started in December 2009 and the study was scheduled to be completed in May 2014. However, the study was terminated two months early on recommendation of the Data Monitoring Committee after a median follow-up of 27 months when analysis of preliminary data demonstrated a highly significant and sustained reduction in the risk of cardiovascular mortality or HF hospitalization (the primary composite end points) in the Sacubitril/Valsartan group compared to the enalapril group (276). At the time of study closure, the available data showed a 20% relative risk reduction in the primary end points, as well as a 16% reduction in all-cause mortality (276). Furthermore, the two major modes of cardiovascular death, sudden death and death from worsening HF, were equally and significantly reduced (279) and both first hospitalizations for HF and total (including repeat) hospitalizations were also reduced (by 21% and 23%, respectively) (280). LCZ696 was also shown to decrease the symptoms and physical limitations of HF to a greater extent than Enalapril (276). There was no statistically significant difference in the rate of angioedema between the groups – although numerically more cases were observed in the Sacubitril/Valsartan group (19 and 10 cases in the
Sacubitril/Valsartan and Enalapril groups, respectively). However, the LCZ696 group had higher proportions of patients with hypotension (14% vs 9% in the in the Sacubitril/valsartan and Enalapril groups, respectively) and non-serious angioedema, but lower proportions with renal impairment, hyperkalemia and cough than the Enalapril group (276). Although concerns have been raised regarding the age of the patients included in this trial (281), subsequent analyses of PARADIGM-HF have concluded that the relative reductions in mortality and morbidity and differential rates of adverse events were similar across all ages (282) and baseline risk of death as determined by the risk-scoring systems Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) and Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) (283). These results show that the relative risk reduction of Sacubitril/Valsartan compared to Enalapril was almost the same as the relative risk reduction of Enalapril compared to placebo (1991) (284). However, given the > 20-year interval between the SOLVD and PARADIGM-HF trials, skepticism to this effect is warranted, especially since the patient populations may not be comparable.

As previously mentioned, the results from the PARADIGM-HF trial led to the approval of Entresto (LCZ696) in the treatment of HFrEF in 2015. Two years later, the American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA) Task Force on Clinical Practice Guidelines published a focused update and the ESC guidelines were also updated – the authors assigned LCZ696 a high class of recommendation (IB) (120) and recommend it as a replacement for ACE-I or ARB in selected patients (120, 263). Although the PARADIGM-HF trial was a large trial that lasted nearly 5 years and provided robust results demonstrating a superior effect of LCZ696 compared to Enalapril on mortality, updating the guidelines based on the results from a single pivotal trial has raised some concerns. For instance, one author has stated that “both guidelines [ESC and ACC/AHA/HFSA guidelines] provide the highest possible recommendation regarding the use of Sacubitril-Valsartan in patients with HFrEF. This is despite the fact that the strength of evidence is not graded at the highest level by either guideline.” (285). Another author raised concerns on possible interpretations based of the findings from the PARADIGM-HF, their implication for clinical care and drawing conclusions based on the results of a single study (286). Intuitively, this seems very reasonable and perhaps use of LCZ696 as a therapeutic agent should be limited until the results from more studies are published. On the other hand, McMurray and colleagues makes an interesting point in a review published shortly after,
stating that “Regulatory approval of a new drug requires demonstration of effectiveness and safety in either two trials with a two-sided $p < 0.05$ OR a single, large, internally consistent, multicenter study with $p < 0.00125$. PARADIGM-HF fulfills these criteria. It was large (8399 patients randomized), highly statistically significant ($p=0.0000004$), internally consistent (lack of subgroup interactions), multicenter (sites were located in 47 countries), and there were large effects on morbidity and mortality (CV death or hospitalization for HF was reduced by 20% and all-cause mortality by 16%). If we ignore the argument that to repeat the trial would be unethical, to achieve such a statistically significant result on the primary end point would require four or five trials each with a $p$ value of $<0.05$ to have the same strength of evidence as provided by a single trial with a $p$ value=$0.0000004$. Put another way, if Sacubitril/Valsartan was in fact no better than enalapril the chances of observing the treatment difference that was found in PARADIGM-HF is less than one in a million.” (287-289).

The results from the PARADIGM-HF trial are arguably sufficiently robust and use of Entresto (LCZ696) in the treatment of selected HFrEF patients, as a replacement for an ACE-I or an ARB, seems both reasonable and, in some cases, favorable.

Other concerns related to Entresto/PARADIGM have been raised regarding the fact that NEP is involved in the metabolism of amyloid-$\beta$ peptide, a key peptide in Alzheimer disease; NEP may block its breakdown to induce Alzheimer disease (290). However, Alzheimer disease and cancer have not been shown to increase with the use of LCZ696 and cognitive decline related to vascular diseases may be reduced by LCZ696.

In conclusion, ARNI (especially LCZ696) has sparked considerable excitement and is the first new HF drug in many years. LCZ696 (Entresto) now has an established role in the treatment of HFrEF and the results from ongoing trials scheduled to be completed this year will determine if Entresto has a role in the treatment of HFpEF and other CV diseases, including hypertension.
4 Discussion

For a stricter adherence to the rules of scientific writing, several issues that were discussed earlier in my thesis could have been discussed in this section instead. However, the relevant issues were discussed when considered relevant and will, for the most part, not be repeated. Instead, we will focus on the limitations of the method (search strategy, selection of data bases, data collection, etc.), discuss possible new approaches to the NPS as a therapeutic target and provide a brief summary and discussion of the main results.

4.1 Method

In hindsight, the search thread I used in my initial search in PubMed could have been better, considering that “heart failure” was listed as a specification in the thread. This probably limited the number of search results and may have excluded some of the studies that have investigated the NPS as a therapeutic target in diseases other than HF. That being said, searching “Natriuretic peptides” in PubMed produces nearly 30,000 results - thus, it was quite challenging to construct a search thread that was neither too specific, nor too general. Another issue regarding the data collection is that the searches were conducted in only 1-2 databases. I also tried to conduct a search in the Cochrane Library using a search thread similar to the one I used in PubMed. However, this produced approximately 1200 results and I simply did not have sufficient time at my disposal to read through an additional 1200 titles and abstracts plus full texts for every abstract that met the eligibility criteria. The additional searches I conducted in PubMed produced several of the same results as the results from my initial search, but also several that were not. This reduced the chance of not including studies relevant to my thesis, but also made the search less systematic.

A possible question from the reader might be why many of the therapeutic agents that target the NPS were not mentioned or described in more detail. Just to be clear; there are several other therapeutic agents targeting the NPS besides those we have discussed. The reason for why many of these agents were not included in my thesis is that they belong to the same groups of therapeutic agents as those included (ARNI, NEP-I, designer NPs etc.). Many of these agents failed to demonstrate desirable effects in early phase clinical trials (and further development was terminated) and/or were inferior to the therapeutic agents included.
4.2 Future approaches to natriuretic peptides as therapeutic agents

Although most studies on therapeutic agents targeting the NPS have focused on their potential role in the treatment of HF (HFrEF, HFpEF, ADHF), the therapeutic potential of NPs also applies to treatment of other pathological states as well. As previously mentioned, hypertension (HTN) and various forms of renal disease (renal failure, CKD; chronic kidney disease, acute tubular necrosis, etc.) are among the conditions in which the NPS has been investigated as a therapeutic target. Clinical studies conducted to date indicate little or no therapeutic benefit of ANP or BNP (analogs) in the treatment of renal diseases (154, 155, 190, 291). However, the use of agents targeting the CNP/NPR-B and/or CNP/NPR-C signal pathway(s) may hold greater potential in the treatment of renal disease than previous studies indicate (see section “Renal disease, NP signal pathways and novel therapeutic agents”). The results from a recent meta-analysis (2017) on LCZ696 (Entresto) in the treatment of HTN indicate that this agent has a greater antihypertensive efficacy than Valsartan and may also have a role in the treatment of HTN (292).

In addition to a possible role in the treatment of renal disease and HTN, the physiological actions of NPs indicate that the NPS represents a therapeutic target in several other diseases as well.

In this section, we will discuss possible new (or rather; unexplored) therapeutic approaches, such as the potential use of NPR agonists and/or positive allosteric modulators (PAMs), and present different aspects important to consider in the search for and development of novel therapeutic agents. We will also discuss pathological states other than HF in which the NPS may represent a therapeutic target.
4.2.1 Novel therapeutic agents

Natriuretic peptide receptor agonists and positive allosteric modulators
Therapeutic agents targeting the NPS can be categorized as either recombinant NPs, designer NPs or inhibitors of NP degradation. However, another possible approach to achieve NP-augmentation is the use of NPR agonists and/or positive allosteric modulators (PAMs).
Despite the development of a vast amount of different types of therapeutic agents targeting the NPS by various mechanisms, this strategy seems to be largely unexplored; no NPR agonists or PAMs have been investigated in clinical trials at the time of writing. Such agents may represent a favorable alternative or supplement to the pharmacological strategies previously attempted, considering that peptides used as drugs have certain drawbacks (e.g. short half-life, usually zero bioavailability when administered per os, etc.).
There is some progress in this field; for instance, a NPR-C antagonist (293) and a series of NPR-A agonists were recently reported (294-296), but these agents have not (yet) been studied in humans/clinical trials and their therapeutic role cannot be determined at this stage.
In addition to more favorable pharmacological properties, non-peptide NPR agonists and PAMs may represent a cost-effective alternative to peptide-based therapeutics as peptide therapeutics cannot be administered per os in most cases and require in-hospital treatment, whereas non-peptide agents are more likely possible to administer by routes other than i.v. and subcutaneous administration. However, identifying non-peptide agents with the ability to activate the NPRs to the same extent as the NPs is a tedious task with a high probability of failure. Nevertheless, several groups are in the process of screening for such agents; some also claim to have identified a series of NPR-A agonists (295, 297) and one group has recently reported the discovery of a NPR-C antagonist (293).
In the case of NPR-A and NPR-B as therapeutic targets, agonists and PAMs are most likely a favorable option (rather than antagonists). The dual clearance and signaling function of NPR-C, however, make it more difficult to draw a priori conclusions on whether an agonist or antagonist is preferable considering that some biological effects mediated by CNP, which indicate a therapeutic benefit, are presumed to result from activation of NPR-C, but NPR-C also has a role in clearing NPs from the circulation and thus reduces the biological effects of the NPs. In any case, the role of NPR-C in neurohormonal modulation is challenging and requires further investigations.
Natriuretic peptides and beta-blockers

Entresto contains both Sacubitril (NEP-I) and Valsartan (ARB) in a single molecule in a 1:1 molar ratio (266). Design of a novel therapeutic agent using the same approach may be of therapeutic benefit; but instead of combining a NEP-I and an ARB, like Entresto, development of a drug containing both an agent targeting the NPS and a β-adrenoceptor antagonist ("beta blocker") could represent a new therapeutic strategy. The rationale for this is the inhibitory effect of the NA/β-AR/AC/cAMP/PKA⁴ signal pathway on NP signaling (the NP/NPR/cGMP/PKG⁵ signal pathway), which involve “cross-talk” that results in reduced efficacy of PKG⁶. Co-activation of these signal pathways (by NPs and noradrenaline) increases the intracellular levels of both cGMP and cAMP and studies have shown that cAMP acts as a partial agonist⁷ on PKG, thus reducing its efficacy (298-301). Addition of a beta-blocker may counteract this inhibitory effect and thus facilitate NP signaling at the cellular level – in other words; co-administration of a therapeutic agent targeting the NPS and a beta-blocker might augment the NPS by both activation of the NPRs, which lead to increased intracellular levels of cGMP and activation of PKG, and counteract the inhibitory effect of cAMP on PKG by blocking the β-adrenoceptor (thus reducing the intracellular levels of cAMP).

In the PARADIGM-HF trial, the patients received Entresto or Enalapril in addition to a beta-blocker (NCT01035255). Combined use of a therapeutic agent targeting the NPS (Entresto) and a beta-blocker might explain, at least in part, why Entresto succeeded in this trial, that is; concomitant use of a beta-blocker may have facilitated NP signaling. β-Blockade may also directly increase NP levels (302, 303). However, in HF patients, NP levels have been shown to be reduced after establishment and up-titration of β-blockade (304, 305). This might represent a barrier for simultaneous use of a beta blocker and a NP-augmenting agent. On the other hand, the underlying mechanisms for reduced NP levels associated with beta blocker-use are not known and does not necessarily exclude a therapeutic benefit of combining such agents (β-blockade and NP augmentation). Besides, the studies that reported reduced NP levels after treatment with a beta blocker also reported that the reduced levels was associated with improvement in cardiac function and functional status.

⁴ NA: Noradrenaline, β-AR: Betaadrenoceptor, AC:adenyl cyclase, cAMP:cyclic AMP, PKA: Protein kinase A
⁵ NP: Natriuretic peptide (ANP, BNP or CNP), NPR: NP receptor (NPR-A or -B), PKG: Protein kinase G
⁶ PKG is the effector molecule of the NP signal pathway and mediates most known effects of NPs
⁷ Partial agonists are drugs/ligands that bind to and activate a given receptor, but have only partial efficacy at the receptor relative to a full agonist.
Considerations for the development of novel therapeutic agents targeting the NPS

Basic research studies have provided great insight into the NPS in general as well as the molecular mechanisms and cellular adaptations that characterizes diseased organs, such as the heart and kidneys. Some of this knowledge is highly relevant and worth considering in future development of novel therapeutics. For instance, both the diseased heart and kidneys are characterized by a shift in NPR expression (NPR-A to NPR-B), that is; the NPR-B receptor is much more prominently expressed in the diseased (failing) heart and kidneys compared to NPR-A and to the expression of NPR-B in non-diseased organs (83, 306-309).

Contrary to common belief, illustrated by the number of agents targeting the ANP/BNP/NPR-A signal pathway that have been developed (most) vs. the number of agents targeting the CNP/NPR-B pathway (close to none), this implies that agents targeting the CNP/NPR-B signal pathway may hold greater therapeutic potential than agents targeting NPR-A. The shift in NPR expression could also partially explain the limited success (i.e. failure) of most agents targeting the NPS; Anaritide, Carperitide, Nesiritide and Ularitide all target the NPR-A receptor and none have demonstrated a beneficial effect on mortality in the treatment of HF patients, whereas only one agent specifically targeting NPR-B have been developed (see BMN111 in the designer NPs section)\(^8\). However, a significant barrier for the development of therapeutic agents targeting the CNP/NPR-B signal pathway is the role of CNP in (stimulating) longitudinal bone growth (endochondral ossification); prolonged activation of NPR-B may lead to unacceptable side effects/adverse events (unwanted bone growth), which severely limits the therapeutic potential of such agents.

Another issue which is important to address is the fact that CNP mainly signals in a paracrine and autocrine manner, whereas drugs administered through traditional routes more closely resemble endocrine signaling. Hence, the right dosage of such agents would be hard to determine since the concentrations of CNP in the tissues are probably much higher than the concentration of CNP in the systemic circulation. Also, it may not be possible to achieve sufficiently high concentrations of the active drug component, which presumably is necessary to reproduce the beneficial effects of CNP, without increasing the risk of unwanted side effects dramatically. Drugs administered through traditional routes are generally distributed throughout the body with almost the same concentrations in the circulation as in the tissues. A possible solution to these problems could be development of new drug delivery systems; for

\(^8\)To the best of my knowledge, the therapeutic effect of BMN111 has not been investigated in HF patients and, based on my results, its therapeutic potential is only being investigated in patients with achondroplasia in an ongoing clinical trial.
instance, therapeutic agents targeting the CNP/NPR-B pathway would probably be much more useful if they could be administered in the form of a capsule (PeptoMicelles or other) coated with antibodies directed at antigens specific to the target organ. This would make it possible to administer drugs at much lower doses and still achieve sufficiently high concentrations at the site of action and, thus, the desired effects, but also relatively low concentrations in the systemic circulation as the drug is distributed (diluted) – which presumably would also reduce the risk of adverse events/unwanted side effects. However, this idea involves the design of a capsule that is able to release the active drug component at exactly the right time, that is; after the capsule is attached to the target organ and before the antibodies anchoring it are degraded. I still think it is a good idea worth sharing, but the solution to this problem I will leave to the hands of more competent individuals.

To summarize, NPR-B is more prominently expressed in the diseased heart and kidneys, which indicates that agents specifically targeting the CNP/NPR-B pathway and/or dual acting agents (able to activate both NPR-A and NPR-B), such as Cenderitide, may hold greater therapeutic potential than agents targeting NPR-A. However, prolonged activation of NPR-B may produce unacceptable side effects that are difficult to avoid, especially by use of traditional drug administration routes, which limits the potential of such agents. A possible solution to this problem is the development of new drug delivery systems/strategies and some new ideas are here needed.

**Multiple NP-augmenting agents**

There are probably good reasons for why no one has investigated combined use of therapeutic agents targeting the NPS, for instance; Entresto and a designer NP vs. Enalapril. One possible explanation is that this may increase the risk of adverse events, such as severe hypotension, angioedema or other, resulting from “over activation” of the NPS. Other reasons could be that it is not possible to patent combined use and therefore not appealing to many researchers or because this approach makes it harder to determine which of the active compounds are effective. In any case, use of multiple agents targeting the NPS might be more effective in the treatment of CV diseases compared to and represent an idea worth exploring. However, no one has investigated this in clinical trials at the time of writing so it is impossible to say whether or not this approach is superior to single-use of an agent targeting the NPS.
4.2.2 The NPS as a therapeutic target in diseases other than HF

Most studies investigating the NPS as a therapeutic target have focused on its potential in the treatment of HF, but several studies have investigated the NPS as a therapeutic target in other diseases as well. However, these studies included few patients in their trials, were of short duration and used surrogate outcomes - although the results may have been encouraging, a therapeutic role of agents targeting the NPS cannot be established on the basis of the results from these studies. Many of the physiological actions of the NPs indicate therapeutic use of agents targeting the NPS in diseases and pathological states besides HF, most notably hypertension (HTN), (post) MI, various kidney diseases and pulmonary hypertension (PH).

Hypertension, NP signal pathways and novel therapeutic agents

Resistant hypertension (RH) is defined as “above-goal elevated blood pressure (BP) in a patient despite the concurrent use of 3 anti-hypertensive drug classes, commonly including a long-acting calcium channel blocker, a blocker of the renin-angiotensin system (angiotensin-converting enzyme inhibitor; ACE-I or angiotensin receptor blocker; ARB), and a diuretic. The anti-hypertensive drugs should be administered at maximum or maximally tolerated daily doses. RH also includes patients whose BP achieves target values on ≥4 anti-hypertensive medications. The diagnosis of RH requires assurance of anti-hypertensive medication adherence and exclusion of the "white-coat effect" (office BP above goal but out-of-office BP at or below target)”, according to the American Heart Association (AHA) (310).

Hypertension is an important risk factor well known to predispose individuals to myocardial infarction (MI), HF, stroke, chronic kidney disease (CKD) and other cardiovascular (CV) diseases (310-312) - these diseases/pathological states are associated with high mortality, severe loss of function and reduced quality of life. Therefore, effective treatment options are paramount in order to prevent the development of these diseases and the complications that often follow. According to the AHA, current management of RH includes “maximization of lifestyle interventions, use of long-acting thiazide-like diuretics (chlorthalidone or indapamide), addition of a mineralocorticoid receptor antagonist (spironolactone or eplerenone), and, if BP remains elevated, stepwise addition of anti-hypertensive drugs with complementary mechanisms of action to lower BP” (310). However, novel therapeutic agents are still needed, especially for patients suffering from both RH and CKD. According to the AHA “Treatment resistance in patients with CKD is undoubtedly related in large part to increased sodium and fluid retention and consequential intravascular volume expansion. An
excess of total body salt and water limits the efficacy of anti-hypertensive medication classes that lack a natriuretic effect.” (310) In other words; a blood pressure (BP-) reducing (anti-hypertensive) agent that concomitantly promote natriuresis and enhance renal function should represent a highly attractive therapeutic option for RH/CKD patients. As previously mentioned, the designer NP “M-ANP” are characterized by its ability to induce BP reduction, promote natriuresis, inhibit aldosterone synthesis and enhance renal function (i.e. increase GFR (238) - Considering its biological effects and the (probable) pathophysiological mechanisms underlying this pathological state (co-morbid RH and CKD), M-ANP has a notable therapeutic potential and a may represent a favorable therapeutic option in the treatment of RH/CKD patients.

RH is also associated with aberrant endothelial function, including loss of nitric oxide (NO-) bioactivity, and increased shear stress resulting in vascular remodeling and dysfunction (313). As mentioned in the introduction, CNP secretion from vascular endothelial cells increases in response to shear-stress (50, 314) and CNP, through activation of NPR-B (probably) or NPR-C, exerts anti-remodeling effects on vascular smooth muscle cells and endothelial cells (315-317) - accordingly, it is plausible that endothelial CNP is protective of vascular insults associated with RH/HTN and that pharmacological interventions targeting this pathway (e.g. CNP-analogs, NPR-B agonists or positive allosteric modulators; PAMs and/or NPR-C agonists or PAMs) may be of therapeutic benefit. However, therapeutic agents targeting the ANP/NPR-A pathway, that is; NPR-A agonists or PAMS, agents that are able to activate both NPR-A and NPR-B (such as Cenderitide/CD-NP) represent an alternative- (or complementary) therapeutic approach in this context, since ANP (NPR-A activation) also has been shown to counteract vascular remodeling through its antihypertrophic and antimitogenic effects on vascular smooth muscle cells and endothelial cells, ability to attenuate the growth response to adrenergic stimuli and prevent migration of endothelial cells (318-322). In fact, a detailed description of the effect of ANP/NPR-A on endothelial cells reveals additional therapeutic potential for agents targeting this pathway (ANP analogs, NPR-A agonists or PAMS, designer NPs; CD-NP, M-ANP, etc.): In a study on the effect of ANP/NPR-A on vascular leakage and angiogenesis (318), the authors concluded that “ANP is an effective inhibitor of VEGF[vascular endothelial growth factor]-induced vascular leakage and angiogenesis in vivo. These results may lead to new treatments for ocular diseases where VEGF plays a central role, such as age-related macular degeneration or diabetic retinopathy.” (318)
The NPs also participate in the chronic regulation of BP through suppression of ACTH (323, 324), vasopressin (323, 325), aldosterone synthesis (ANP only) (326) and sympathetic outflow (323, 327, 328)- thus, therapeutic agents targeting signal pathways involving ANP/BNP/NPR-A, CNP/NPR-B and/or NPR-C may represent a future approach to exploit the NPS in the treatment of HTN and RH.

Other biological effects involving different NP signal pathways also represent therapeutic targets that may be beneficial in the treatment of HTN/RH: Administration of exogenous CNP induces an acute drop in BP in anesthetized dogs and humans with chronic renal failure (CRF) (329, 330) - this supports the potential use of therapeutic agents targeting the CNP/NPR-B (or NPR-C) pathway, in the treatment of HTN and concomitant renal disease. Low dose infusions of ANP reduces peripheral vascular resistance and BP in humans, but at higher doses the reduced BP is accompanied by increased peripheral vascular resistance; probably due to the activation of counter regulatory hormones, such as the RAAS (331). This suggests a dose-dependent potential for agents targeting the ANP/NPR-A pathway as antihypertensive agents, but undesirable effects at higher doses limit their therapeutic potential as antihypertensive agents and the CNP/NPR-B (or NPR-C) pathway may represent a favorable therapeutic target.

BNP infusions cause no significant changes in BP or heart rate when administered to hypertensive or normotensive human subjects in doses resulting in plasma concentrations comparable to the plasma levels of BNP in HF patients (331). However, BNP infusions in congestive HF (CHF) patients, administered in higher doses, have been shown to reduce systemic vascular resistance (SVR), BP and pulmonary capillary wedge pressure (PCWP), increase stroke volume, promote natriuresis and diuresis and reduce aldosterone levels (24). All these biological effects indicate a therapeutic role for agents targeting the BNP/NPR-A pathway (BNP analogs, NPR-A agonists or PAMs, etc.) in the treatment of hypertensive states - however, these results also suggest that the clinical use of such agents might be limited to treatment of hypertensive states in CHF patients (considering that the BP lowering actions of BNP were only seen in CHF patients, whereas similar effects were not seen in normotensive and hypertensive patients)
Myocardial infarction, cardiac remodeling, NP signal pathways and novel therapeutics

Myocardial infarction (MI) is defined pathologically as myocardial cell death due to prolonged ischemia (332). This causes diminished cellular glycogen, relaxed myofibrils and sarcolemmal disruption and mitochondrial abnormalities as early as 10-15 minutes after the onset of ischemia (333, 334).

It can take hours before myocyte necrosis (cell death) can be identified by postmortem examination in humans (332). Timely implementation of reperfusion therapy (i.e. restoration of blood flow) reduces ischemic injury of the myocardium (335, 336).

Reperfusion of ischemic tissues is essential to limit the extent of myocardial necrosis and preserve organ function in MI. However, reperfusion also exerts detrimental effects by promoting cell death, inflammation and microvascular dysfunction; this phenomenon is known as ischemia/reperfusion (I/R) injury (337). I/R injury is also associated with loss of endothelium-derived mediators, such as nitric oxide (NO), which results in vasoconstriction, decreased perfusion, recruitment of leukocytes and platelet activation - which combined exacerbate damage (335).

The results from several preclinical studies indicate that the CNP/NPR-B and/or CNP/NPR-C signal pathway(s) may offer protection from I/R injury; for instance, one study has shown that ventricular hypertrophy, necrosis, inflammation and functional impairment of cardiac tissue induced by coronary artery ligation is reduced in mice over-expressing CNP in cardiomyocytes (338). Another (preclinical) study demonstrated that CNP infusion administered post MI attenuates cardiac fibrosis and left ventricular hypertrophy and increase survival in male Sprague-Dawley rats (107). CNP has also been shown to down-regulate the expression of many pro-hypertrophic genes and transcription factors, including MEF2 and GATA4 and opposes the hypertrophic effects of endothelin-1 and angiotensin II (105, 339, 340).

Furthermore, CNP has been shown to reduce infarct size following I/R injury in isolated rat hearts, most likely via the NPR-C (non-cGMP-mediated) signal pathway (341); NPR-C activation seems to underlie the salutary effects of CNP on the myocardium and in regulating coronary blood flow - thus, administration of therapeutic agents targeting the CNP/NPR-C pathway post-MI could be of therapeutic benefit (i.e. limit infarct size and cardiac remodeling, etc.). This claim is also supported by observations of enhanced bioactivity of CNP in the absence of endogenous NO production, which indicate that CNP may play a compensatory role in protecting the heart and vasculature when NO signaling is impaired (342).
Renal disease, NP signal pathways and novel therapeutic agents

Despite the potent natriuretic and diuretic effects of NPs (ANP, BNP; NPR-A) in healthy subjects, clinical studies conducted to date indicate little or no therapeutic benefit of NP analogs in the treatment of renal disease (154, 155, 190, 291) However, the use of therapeutic agents targeting the CNP/NPR-B pathway may hold greater therapeutic potential than previous studies indicate.

A possible explanation to the attenuated renal response to ANP/BNP/NPR-A in patients with kidney disease compared to the renal effects in response to NPs in healthy volunteers (natriuresis, diuresis, increased GFR, etc.) might be chronic upregulation of NPR-C in the renal cortex and/or downregulation of NPR-A in the renal medulla in pathological states affecting the kidneys (156, 343-345). On the other hand, several studies have shown that the expression of CNP is often up-regulated in several pathological conditions affecting the kidneys, such as nephrotic syndrome, renal failure, diabetic nephropathy and unilateral uretral obstruction (306-309), and that high levels of CNP is associated with-, and may contribute to, increased expression of NPR-B in renal tissues (346, 347). The CNP/NPR-B (and/or NPR-C) signal pathway mediate several beneficial physiological actions, such as inhibition of interstitial fibrosis and vascular remodeling (306, 308, 347), which indicate a therapeutic potential for agents targeting this signal pathway in the treatment of various types of kidney disease. However, there are no therapeutic agents that specifically target the CNP/NPR-B pathway available for use in clinical trials so the therapeutic role of such agents will remain a mystery.
Pulmonary hypertension, NP signal pathways and novel therapeutic agents

Primary pulmonary hypertension (PH) is a pathological state characterized by increased pulmonary artery pressure, remodeling of the pulmonary resistance vasculature and right ventricular hypertrophy, eventually leading to right HF and death (348-350). Current therapeutic options include prostacyclin analogs (351-353), endothelin receptor antagonists (354-356) and PDE5 inhibitors (357-360), all of which have been shown to improve symptoms, exercise capacity and hemodynamics, however, the long-term prognosis remains poor (361, 362) and PH represents a clear unmet medical need.

The pathophysiology of PH involves reduced bioactivity of NO and other endothelium-dependent vasodilators, such as prostacyclin (350, 364, 365), and increased production of endogenous vasoconstrictors such as endothelin-1 (ET-1) (366). Furthermore, this pathological state is characterized by pulmonary endothelial dysfunction which precipitates excessive pulmonary vasoconstriction and remodeling (342). The strategy of elevating cGMP by augmenting NO-dependent signaling (e.g. NO inhalation, direct soluble guanylyl cyclase; sGC stimulation and PDE5 inhibitors) is clinically effective in PH (367, 368). As previously discussed, activation of NPR-A and NPR-B also increases the intracellular levels of cGMP. This provides rationale for use of therapeutic agents targeting the NP signal pathways instead of- or in addition to traditional therapeutics. However, the results from two studies indicate that NP analogs are not more effective in the treatment of PH than PDE5 inhibitors alone, but has an additive effect when administered with a PDE5 inhibitor (e.g. sildenafil) (363, 369). Inhibitors of neutral endopeptidase (NEP-I) augment NP bioactivity and produce a selective pulmonary dilation; this mechanism also reduces pulmonary vascular remodeling and right ventricular hypertrophy (363). Furthermore, altering CNP signaling may also prove therapeutically effective. This idea is supported by the results from a study which demonstrated that CNP ameliorates the development of PH in an animal model (370).

However, other studies have reported that CNP is less important than ANP or BNP in protecting against hypoxic PH in rats and that the BNP/NPR-A signal pathway probably represents a more favorable therapeutic target (371, 372). NPR-C has also been suggested as a potential target in the treatment of PH (373), but there is limited data supporting this claim. To summarize, augmentation of the NPS by the use of NEP-I and/or BNP analogs may represent a therapeutic option in the treatment of PH, especially if combined with a PDE5 inhibitor. However, the NPS seems to hold greater therapeutic potential in the treatment of other diseases.
4.3 Summary of results

Since the discovery of the natriuretic peptide system (NPS) nearly 40 years ago, the NPS has been regarded as an attractive therapeutic target for treatment of cardiovascular (CV) and renal diseases, especially heart failure. One of the reasons the NPs/NPS is believed to hold great therapeutic potential is their intrinsic biological effects (reduced BP, anti-remodeling effects, increased GFR, etc.).

Numerous investigators have attempted to target the NPS in the treatment of HF and other diseases and several therapeutic agents have been developed. All of these agents augment the NPS by various mechanisms and can be categorized as either recombinant NPs, designer NPs, inhibitors of NP degradation (or other).

Recombinant NPs (Anaritide, Carperitide, Nesiritide, Ularitide) represent the first attempts to target the NPS in the treatment of HF. The recombinant forms of NPs are structurally and functionally identical to their endogenous counterpart and two forms of recombinant agents (recombinant ANP and BNP; Carperitide and Nesiritide, respectively) was approved for use in the treatment of acute decompensated heart failure (ADHF) in 1995 and 2001 in Japan and the USA, respectively.

Shortly after the initial investigations into the use of recombinant agents as therapeutics, several therapeutic agents that can be classified as neprilysin inhibitors (NEP-I) and later Vasopeptidase inhibitors (ACE/NEP-I9) were developed and investigated in preclinical studies and Phase I and II clinical trials.

Therapeutic use of NPs was initially hampered by limited clinical efficacy (probably due to rapid enzyme degradation) and severe adverse events, such as excessive hypotension, angioedema and anemia, which terminated further clinical development of these agents.

Progress in the field of NP research was made by the introduction of designer NPs (CD-NP, CU-NP, ANX-042, M-ANP, etc.) and was further advanced by the concept of angiotensin receptor neprilysin inhibitors (ARNI), in particular LCZ696 (trade name: Entresto; Sacubitril/Valsartan). Designer peptides are engineered (chimeric) peptides produced by combining various segments of native NPs (Fig 5. and Fig 6.). Overall, current designer NPs are more efficacious in their actions than their predecessors (recombinant NPs). However, these agents are still in the early stage of development and, like their predecessors, have demonstrated beneficial effects only on surrogate outcomes in Phase I and II clinical trials.

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9 ACE/NEP-I = Angiotensin converting enzyme neprilysin inhibitors
No phase III trials investigating their effect on mortality and other important clinical outcomes have been completed at the time of writing. Thus, it remains to be seen if the effects observed in preclinical studies, healthy volunteers and phase I and II clinical trials will translate into an effect on mortality and other important clinical outcomes in larger Phase III trials. Based on the results from previous studies on recombinant NPs and, with the exception of Entresto, all inhibitors of NP degradation, one cannot rule out that designer NPs will share the fate of their predecessors – “promising results” in small, early phase clinical trials of short duration followed by disappointment in larger phase III clinical trials seems to be a regular pattern when reviewing the history of the NPS as a therapeutic target. A possible reason for why so many agents have shown encouraging results in the early phase of clinical trials, but fail to demonstrate an effect on mortality and other important outcomes in larger Phase III trials, could be that the duration of the treatment needs to be longer in order to produce demonstrable, significant effects on mortality. Other reasons have been suggested and include short duration of infusion time (24–48 h), fixed treatment dose regimen, protocol violations and the failure to use guideline- directed therapy in the majority of Phase 2 trials. The limited success (i.e. failure) of most therapeutic agents targeting the NPS, especially those targeting the ANP/BNP/NPR-A signal pathway, could also be partially explained by a shift in NPR expression from NPR-A to NPR-B. Thus, the CNP/NPR-B (and/or NPR-C) signal pathway(s) may represent a favorable therapeutic target compared to the ANP/BNP/NPR-A signal pathway. However, the role of CNP/NPR-B in longitudinal bone growth increases the risk of unacceptable side effects (unwanted bone growth), which severely limits the therapeutic potential of such agents. A possible solution to this problem could be development of new drug delivery strategies, for instance a capsule (PeptoMicelles or other forms) coated with antibodies specific to the target organ that is able to release the active drug component at the site of action), however, no such delivery systems exists and may not be possible to develop.

To date, only one of the many therapeutic agents targeting the NPS has shown an effect on mortality greater than- or equal to therapeutic agents recommended for treatment of HFrEF; Entresto (LCZ696). Entresto (LCZ696) was registered in Europe in 2015 and is recommended for use in the treatment of selected HFrEF patients according to the ESC 2016 guidelines (120) and ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure (263). Its potential role in the treatment of HF with preserved ejection fraction (HFpEF) is currently under investigation in a large, phase III clinical trial (PARAGON-HF), which is estimated to be completed on May 31, 2019.
Entresto was approved for clinical use (i.e. treatment of selected HFrEF patients) after the results from the PARADIGM-HF trial were published in *The New England Journal of Medicine* in 2014. This trial demonstrated a relative risk reduction of approximately 20% on CV death and hospitalization in the Entresto-group compared to the Enalapril group. The trial was stopped early due to the overwhelming evidence of the mortality-reducing effect of this agent compared to Enalapril. There are several ongoing trials investigating the role of Entresto and designer NPs in the treatment of various diseases – however, it remains to be seen if the therapeutic role of these agents is as prominent as suggested by the developers and several reviewers.
5 Conclusion

Several therapeutic agents targeting the NPS have been developed since this group of signal peptides emerged nearly 40 years ago. Although several of these agents were considered successful in treatment of HF for a long time, all (with the exception of LCZ696/Entresto) were later shown to lack efficacy and/or were associated with severe side effects. The assumption that these agents were successful in treating HF patients was largely based on results from phase I and II clinical trials using surrogate outcomes, such as various hemodynamic parameters (PCWP, SVR, etc.), biomarkers (NT-proBNP) and others. In almost all cases, however, these outcomes did not translate into beneficial effects on mortality, morbidity or other important clinical outcomes or the “promising results” were not reproduced in larger Phase III clinical trials. In fact, “promising results” in early phase clinical trials followed by disappointing results in larger Phase III trials seems to be a regular pattern when reviewing the NPS in a historical setting. This is especially true for Nesiritide - a recombinant form of BNP long considered a novel therapeutic agent suitable for use in HF therapy. However, the results from the ASCEND-HF trial, the largest study investigating the therapeutic role of Nesiritide conducted to date, effectively put an end to this misconception and the production of Nesiritide was recently discontinued. Several novel therapeutic agents considered to hold great therapeutic potential have been developed in recent years, most notably a group of agents known as “designer NPs”. These agents have shown promising results in “proof-of-concept”-studies and considered to hold great therapeutic potential, but their role in future treatment of disease has not yet been established.

“Therapeutic potential” seems to be a recurring phrase in the literature. However, only one therapeutic agent targeting the NPS (and other endocrine systems) has an established role in treatment of disease; Entresto (LCZ696). This first-in-class ARNI was registered in Europe in 2015 and is recommended for treatment of selected HFrEF patients according to the ESC- and ACC/AHA/HFSA guidelines. The success of Entresto has, indeed, been attributed to NP augmentation. However, the underlying mechanisms responsible for its mortality-reducing effects compared to Enalapril are still unclear and the extent of the contribution of the NPS is debatable. There are several good arguments that support a prominent role of the NPS in HF therapy, but the extent of its contribution may also be exaggerated. Nevertheless, therapeutic use of NPs remains a highly sought after goal and the era of NPs as therapeutic agents continues to evolve with the promise of exciting therapeutics for CV and other diseases.
References


Appendix

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>aa</td>
<td>Amino acid(s)</td>
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<tr>
<td>AC</td>
<td>Adenylyl cyclase</td>
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<td>ACE/NEP-I</td>
<td>Angiotensin converting enzyme neprilysin inhibitors (aka Vasopeptidase inhibitors)</td>
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<td>ADHF</td>
<td>Acute (decompensated) heart failure</td>
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<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide (aka atrial natriuretic factor)</td>
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<tr>
<td>AngII</td>
<td>Angiotensin II</td>
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<tr>
<td>ARB</td>
<td>Angiotensin II (type 1) receptor blocker</td>
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<tr>
<td>ARNI</td>
<td>Angiotensin receptor neprilysin inhibitor</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>BNP</td>
<td>B-type natriuretic peptide (aka brain natriuretic peptide)</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CHF</td>
<td>Chronic heart failure</td>
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<tr>
<td>CNP</td>
<td>C-type natriuretic peptide</td>
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<td>CV</td>
<td>Cardiovascular</td>
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<tr>
<td>DNP</td>
<td>Dendroaspis natriuretic peptide</td>
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<tr>
<td>cAMP</td>
<td>3',5'-cyclic adenosine monophosphate</td>
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<tr>
<td>cGMP</td>
<td>3',5'-cyclic guanosine monophosphate</td>
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<tr>
<td>GC</td>
<td>Guanylyl cyclase</td>
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<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
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<tr>
<td>HF</td>
<td>Heart failure</td>
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<td>HFrEF</td>
<td>Heart failure with reduced ejection fraction (aka systolic HF)</td>
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<td>HFP EF</td>
<td>Heart failure with preserved ejection fraction (aka diastolic HF)</td>
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<tr>
<td>HTN</td>
<td>Hypertension</td>
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<tr>
<td>LV(EF)</td>
<td>Left ventricular (ejection fraction)</td>
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<tr>
<td>M-ANP</td>
<td>Mutant atrial natriuretic peptide or M-atrial natriuretic peptide</td>
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<tr>
<td>MI</td>
<td>Myoccardial infarction</td>
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<tr>
<td>MRA</td>
<td>Mineralocorticoid receptor antagonists</td>
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<tr>
<td>NEP(-I)</td>
<td>Neutral endopeptidase (aka neprilysin; NEP 24.11) (NEP-I; NEP-inhibitors)</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>NPPA</td>
<td>Natriuretic precursor peptide A (-B, -C)</td>
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<tr>
<td>NPR</td>
<td>Natriuretic peptide receptor (A, B; aka particulate guanylyl cyclase; pGC-A, pGC-B)</td>
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<tr>
<td>NP(s)/NPS</td>
<td>Natriuretic peptide(s)/(The) Natriuretic Peptide system</td>
</tr>
<tr>
<td>PAMs</td>
<td>Positive allosteric modulators</td>
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<tr>
<td>PH</td>
<td>Pulmonary hypertension</td>
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<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
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<tr>
<td>PDEs</td>
<td>Phosphodiesterases</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein Kinase A (or cAMP dependent protein kinase)</td>
</tr>
<tr>
<td>PKG</td>
<td>Protein Kinase G (or cGMP dependent protein kinase)</td>
</tr>
<tr>
<td>RAAS</td>
<td>The renin-angiotensin-aldosterone system</td>
</tr>
<tr>
<td>SNS</td>
<td>The sympathetic nervous system</td>
</tr>
<tr>
<td>SVR</td>
<td>Systemic vascular resistance</td>
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<tr>
<td>URO</td>
<td>Urodilatin</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>VNP</td>
<td>Vasonatrin</td>
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<tr>
<td>Target organ</td>
<td>Physiological action</td>
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<td>-------------</td>
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</tbody>
</table>
| Heart       | ▪ ↓ Preload, ↓ afterload, ↓ cardiac output  
              ▪ (↑) Lusitropic response  
              ▪ ↓ Cardiac remodeling (hypertrophy, fibrosis) |
| Vasculature | ▪ ↓ Blood pressure, ↓ blood volume  
              ▪ Vasodilation (arteries, veins, arterioles)  
              ▪ ↓ Vascular remodeling (inhibition of vascular smooth muscle cell proliferation, fibroblast proliferation and extracellular matrix production)  
              ▪ ↑ Capillary hydraulic conductivity |
| Kidney      | ▪ ↑ Glomerular filtration rate (GFR) by inducing vasodilation in afferent arterioles and vasoconstriction in efferent arterioles  
              ▪ ↑ Natriuresis through inhibition of the Na⁺/H⁺-exchanger in the proximal tubule, the Na⁺/Cl⁻ co-transporter in the distal tubule and Na⁺ channels in the collecting duct  
              ▪ ↑ Diuresis through inhibition of vasopressin-induced aquaporin 2-incorporation into the apical membrane of the collecting ducts |
| Neuroendocrine | ▪ Suppression of the RAAS, SNS (sympathetic outflow), vasopressin (aka AVP and ADH), ACTH (in the CNS) and endothelin |
| Other       | ▪ Bone growth: regulation of endochondral ossification (CNP only)  
              ▪ Neuronal development, neuroprotection and reproduction |

Table 1. The NPs mediate a diverse array of biological effects, many of which are listed in this table. The NPs are important regulators of fluid and electrolyte balance and also help preserve structural integrity of the heart and vasculature through their anti-remodeling actions.

Abbreviations: ACTH; Adrenocorticotropic hormone, AVP; arginine vasopressin (aka vasopressin or ADH; antidiuretic hormone), CNP; C-type natriuretic peptide, RAAS; (the) renin-angiotensin-aldosterone system, SNS; sympathetic nervous system.

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10 Different NPs mediate different biological effects. For instance, ANP and BNP promote natriuresis and diuresis, whereas CNP has limited renal effects. However, CNP is the only NP involved in bone growth.
Fig 1. Structure of the natriuretic peptides

All NPs contain a 17 amino acid (aa) ring linked by a disulfide bond between two cysteine residues within their structure. Although the presence of DNP in humans is controversial, especially since no gene encoding DNP has been identified in humans, it is still included in this figure to illustrate the structural similarity between the NPs.
Fig 2. All NPs are the product of three separate genes; NPPA, NPPB and NPPC (located on chromosome 1 and 2). Transcription of these genes produces the prepropeptides preproANP, preproBNP or preproCNP. Enzymatic cleavage of the prepropeptides removes a signal peptide sequence, which results in the formation of proANP, proBNP or proCNP. These propeptides are further processed by removal of the N-terminal segments to produce mature, biologically active, natriuretic peptides (ANP-28, BNP1-32, BNP4-32, CNP-53 and CNP-22) and biologically inactive N-terminal propeptide segments (NT-proANP, NT-proBNP and NT-proCNP). Corin, Furin and other enzymes (some unknown) are responsible for the enzymatic processing during synthesis, whereas NEP, DPP4 and IDE breaks down the NPs through enzymatic degradation, as shown in this figure and fig 3. The N-terminal segment of proANP contain several peptides with natriuretic and diuretic effects called vessel dilator, kaliuretic peptide and long-acting natriuretic peptide or LANP (not shown in figure). However, these peptides lack the typical structure of NPs, although they elicit similar biological effects.

Abbreviations: NPPA (-B, -C); natriuretic peptide precursor A (-B, -C), NT-proANP (BNP/CNP); N-terminal proANP (BNP/CNP), NEP; Neprilysin; neutral endopeptidase (24.11), IDE; Insulin degrading enzyme, DPP4; Dipeptidyl peptidase-IV
Fig 3. NP signal pathways and primary clearance mechanisms

Fig 3. The natriuretic peptides elicit their biological effects by binding to NPR-A, NPR-B or NPR-C. NPR-A and NPR-B are guanylyl cyclase-coupled receptors and ligand-binding to these receptors induces a conformational change that catalyze the intracellular conversion of GTP to cGMP. cGMP activates PKG, which mediates most known biological effects of NPs, and is inactivated by intracellular PDEs.

NPR-C bind all NPs with high affinity and clears them from the circulation. Ligand-binding to this receptor results in receptor-mediated internalization and lysosomal degradation (transforming the NPs to inactive products). NPR-C also has a signaling function and is believed to signal through G\textsubscript{i}-protein mediated inhibition of the AC/cAMP/PKA pathway and interact with PLC to produce non-cGMP-mediated biological effects. The NPs are also cleared from the circulation through enzymatic degradation by NEP, IDE and DPP4 (and possibly other enzymes as well).

Abbreviations: AC; adenylyl cyclase, ANP; Atrial Natriuretic Peptide, ATP; adenosine triphosphate, BNP; B-type Natriuretic Peptide, cAMP; 3’5’ cyclic-adenosine monophosphate, cGMP; 3’5’-cyclic guanosine monophosphate, CNP; C-type Natriuretic peptide, DAG; diacylglycerol, DNP; Dendroaspis Natriuretic Peptide, DPP4; Dipeptidyl peptidase-IV, GC; guanylyl cyclase (aka guanylate cyclase), GMP; guanosine monophosphate, GTP; guanosine triphosphate, NPR; Natriuretic peptide receptor (–A, -B, -C), IDE; Insulin degrading enzyme, IP3; Inositol trisphosphate (or inositol 1,4,5-trisphosphate), NEP; Neprilysin; neutral endopeptidase (24.11), PDEs; Phosphodiesterases, PKA; protein kinase A (aka cAMP dependent protein kinase), PKG; protein kinase-G (aka cGMP-dependent protein kinase), PLC; Phospholipase C
**Fig 4:** Recombinant NPs; their structure and amino acid sequences

![Recombinant NPs Diagram](image)

**Fig 4.** Recombinant NPs are structurally identical to their endogenous counterpart (native NPs) and have the same biological properties (biological effect, plasma half-life, receptor profile, etc.)

**Fig 5.** Designer natriuretic peptides

![Designer NPs Diagram](image)

**Fig 5.** The structure of some of the designer NPs developed by investigators at the Mayo clinic. Although the structure of designer NPs as marginally different from the structure of native NPs, these agents have unique properties that may have therapeutically beneficial effects.
Fig 6: Designer NPs - Structure and synthesis of Cenderitide

*Fig 6.* Cenderitide (CD-NP) is a hybrid (chimeric) peptide produced by combining segments of CNP and DNP. This gives the peptide unique properties and more desirable effects compared to native NPs.