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BIOCHEMISTRY, THERAPEUTICS AND BIOMARKER IMPLICATIONS OF NEPRILYSIN IN CARDIORENAL DISEASE

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Abstract

Background: Neprilysin (NEP) is a membrane bound neutral endopeptidase which degrades a variety of bioactive peptides. The substrates include natriuretic peptides (NPs), which are important regulating mediators for cardiovascular and renal biology. Inhibiting NEP activity and exogenous NP administration thus have emerged as potential therapeutic strategies for treating cardiorenal diseases. More recently, B-type natriuretic peptide (BNP) or N-terminal-proBNP (NT-proBNP), 3'−5' cyclic guanosine monophosphate (cGMP) and soluble NEP as biomarkers have also been investigated in heart failure (HF) trials and their predictive value are beginning to be recognized.

Content: The biological functions of NEP and NPs are discussed. Enhancing NPs through NEP inhibition combined with renin-angiotensin-aldosterone system (RAAS) antagonism has proved to be successful in HF treatment although future surveillance studies will be required. Direct NP enhancement through peptide delivery may have fewer potentially hazardous effects compared to NEP inhibition. Strategies of combined inhibition on NEP with other cardiorenal pathophysiological pathways are promising. Finally, monitoring BNP/NT-proBNP/cGMP concentrations during NEP inhibition treatment may provide supplemental benefits to conventional biomarkers, and the identification of soluble NEP as a novel biomarker for HF needs further investigations.

Summary: In this review the biology of NEP is summarized, with a focus on NP regulation. The degradation of NPs by NEP provides the rationale for NEP inhibition as a strategy for cardiorenal disease treatment. We also describe the current therapeutic strategies of NEP inhibition and NP therapeutics in cardiorenal diseases. Moreover, the discovery of its circulating form, soluble NEP, as a biomarker is also discussed in the review.

Keywords

Neprilysin; natriuretic peptides; neprilysin inhibition; heart failure; therapeutics; biomarker

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BIOCHEMISTRY OF NEPRILYSIN AND NEPRILYSIN INHIBITION

Biochemistry of Neprilysin:

Neprilysin (NEP, neutral endopeptidase, enkephalinase, E.C.24.11) was discovered from rabbit kidney proximal tubule brush border membranes by the Kerr and co-workers (1). It is a zinc-dependent membrane metallopeptidase with a subunit molecular weight (Mr) of 90 kDa and contains glycosylation sites (2). NEP is highly conserved among mammals, with strong similarity between rat and rabbit and only a six amino acid (AA) difference in sequences between human and rat. NEP belongs to the M13 subfamily of neutral endopeptidases and consists of a short intracellular N-terminal domain, a single transmembrane helix, and a large C-terminal extracellular domain (3). The enzyme active site is located in the C-terminal extracellular domain.

The crystal structure of the extracellular domain (residues 52–749) of human NEP bound to the its inhibitor phosphoramidon at 2.1 Å resolution revealed that extracellular NEP exists as two multiply connected folding domains which embrace a large central cavity containing the active site (3). The selectivity of NEP substrates limited to 3000 Da (3) probably results from the molecular sieving function of domain 2, which restricts the active site access by larger peptides. This may partly explain why larger NPs such as dendroaspis NP (DNP), CD-NP (Cenderitide) and mutant atrial NP (MANP) are poor substrates for NEP (4-6).

NEP Substrates:

NEP is widely distributed in various tissues which include kidney, lung, brain, heart, and vasculatures. Importantly, the kidney is the richest source which was identified with the use of a NEP monoclonal antibody in porcine renal tissues (7). A critical property of NEP is that it cleaves and degrades a variety of bioactive peptides (Table 1). From this perspective, NEP has high relevance to cardiovascular and renal regulation and to understand the modulations of these substrates by NEP is critical for understanding therapeutic as well as diagnostic implications.

NEP cleaves peptides at the amino side of hydrophobic residues (e.g. Phe, Leu, Tyr, Trp) and was previously given the name enkephalinase as it hydrolyzes enkephalin at its Gly3-Phe4 bond. Extensive work has focused on the NPs as they may play a key role in the therapeutics of NEP inhibition. Studies have established that the cleavage sites of human Atrial NP (hANP) are: Cys7-Phe8, Arg4-Ser5, Arg11-Met12, Arg14-Ile15, Gly16-Ala17, Gly20-Leu21, and Ser25-Phe26 with Cys7-Phe8 as the primary cleavage site (8). Human B-type NP (hBNP) cleavage sites are: Met4-Val5, Arg17-Ile18 (8). Human C type NP (hCNP) cleavage sites are: Cys6-Phe7, Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17, Gly19-Leu20 (8). Another NP, urodilatin (URO), may present a similar degradation pattern as ANP with initial cleavage site at the Cys11-Phe12 (9). It should be noted that the addition of 4 AA to the N-terminus of ANP which forms URO renders URO more resistant to degradation. The open ring structure of ANP and CNP by NEP cleavage at Cys-Phe leads to the loss of activity.

NEP and the NP System:

From a biological perspective, the degradation and clearance of NPs have been implicated as a critical regulatory pathway controlling sodium, water balance and blood pressure homeostasis which has prompted interest in NEP inhibitors as a therapeutic strategy to potentiate native NPs. Sonnenberg et al compared the degradation products by rat NEP and kidney cortex membrane to identify the major degrading enzyme of rat ANP (rANP). They reported that ANP cleavage by NEP produces a major hydrolytic product that is consistent with kidney cortex membrane degradation (10). Thus they concluded that NEP is the major degrading enzyme for NPs, particularly ANP. Compared to murine ANP degradation by wild type mice kidney membranes, incubation with the NEP inhibitor candoxatrilat and membranes from NEP deficient mice resulted in substantial delay in ANP degradation (11). Consistent with the rich distribution of NEP in the kidney, whole body radioautography in rats revealed that the kidney is the predominant organ involved in hANP metabolism and clearance (12). Another NP, CNP, is also a preferred substrate for NEP in which hCNP is quickly degraded (8). In contrast, BNP is more resistant to NEP degradation (8). In vitro kinetic data for hANP, hBNP, hCNP incubated with purified human NEP was: (K_{cat}/K_m) CNP 7.85 > ANP 5.12 > BNP 0.53 (8). Other studies have also reported that hBNP is a poor substrate of NEP (13, 14). Rodent BNP has also been reported to be resistant to NEP degradation (11, 15, 16). However, future studies to compare the difference in catabolism between rodent and human BNP are needed. Similar to what was observed with BNP, URO is degraded relatively slower by NEP than ANP (9, 17). In an experiment in which URO and hANP were incubated with NEP, URO remained intact or was partially degraded while most of the ANP was degraded (17). Such data may provide insight into which NPs may be contributing to therapeutic outcomes with NEP inhibition.

NEP Inhibition:

NEP inhibitors have been developed which show enhanced diuretic and natriuretic actions through NPs and the second messenger cGMP particularly in the setting of experimental HF in which endogenous ANP and BNP are increased. Previously our laboratory reported that chronic oral NEP inhibition by candoxatril delays the onset of reduction in Na^+ excretion, enhances ANP activity, and suppresses aldosterone activation in experimental HF (18). The NEP inhibitior candoxatril in human chronic HF increased plasma ANP levels, promoted natriuresis and diuresis (19). Thus, NEP inhibitors may supplement conventional HF treatment.

Importantly, given that many substrates for NEP are peptides with vasoactive as well as neuro-regulatory properties, inhibiting NEP could potentially result in undesirable adverse effects. For example, Amyloid β (Aβ plays a central role in Alzheimer's disease pathology) is a known substrate for NEP in the brain where NEP is also distributed. The possibility of NEP inhibitors, especially small molecules, crossing the blood-brain barrier in patients with cardiovascular diseases raises concerns regarding NEP inhibition in treating HF patients. Thus, surveillance studies are warranted with ongoing NEP inhibition in human disease (20). In addition, NEP (or common acute lymphoblastic leukemia antigen, CD10) is involved in tumor cell proliferation and extracellular matrix structure regulation, which raises the question of possible multifaceted functions either inhibiting or enhancing tumor

development. Studies have shown that NEP inhibits initiation or progression of small cell carcinoma of the lung or may enhance metastasis of colorectal cancer (21, 22). Thus inhibition of NEP could also modulate the risk for oncogenesis or cancer outcomes also warranting surveillance strategies.

TARGETING NEPRILYSIN AS A THERAPEUTIC STRATEGY FOR HEART FAILURE

Selective Neprilysin Inhibition:

With the realization that the NPs are a major substrate for NEP inhibition, their biology has been the focus of extensive investigation with selective NEP inhibitors. As illustrated in Figure 1 the NPs target particulate guanylyl cyclase receptors A (pGC-A, natriuretic peptide receptor A/NPRA) and B (pGC-B, natriuretic peptide receptor B/NPRB). Through activation of cGMP and the downstream signaling pathways, NPs mediate widely pleotropic beneficial actions. To date, NEP inhibition clearly potentiates the biological actions of NPs such as ANP. In mild experimental HF in which the renin-angiotensin-aldosterone system (RAAS) is not activated, NEP inhibition was natriuretic and cardiac unloading (23). In contrast, in severe experimental HF in which the RAAS was activated, NEP inhibition resulted in less natriuretic actions providing initial insights in to the need to co-target the RAAS in the presence of inhibition of NEP (23). In human studies, the importance of also targeting the RAAS was the report that candoxatril also increased circulating Angiotensin II (ANG II) (24). In human chronic HF, candoxatril treatment increased both ANP and BNP, augmented diuresis and natriuresis and reduced clearance of exogenously administered ANP (19). Finally, although plasma NPs were increased and there was an enhanced renal response, systemic and pulmonary vascular resistances were not reduced by candoxatrial in human HF which was consistent with observations in experimental HF in which NEP inhibition was natriuretic and aldosterone suppressing without hemodynamic benefit (18).

Dual Inhibition of NEP and Angiotensin Type 1 Receptor Antagonism:

Based on the findings that RAAS over-activation attenuates the renal actions of NEP inhibition as well as the observation of increases in circulating ANG II with NEP inhibition, the concept of dual inhibition of NEP with simultaneous angiotensin type 1 (AT1) receptor antagonism emerged. Margulies et al in experimental HF established that ANG II inhibition with an angiotensin converting enzyme inhibitor (ACEI) potentiated the glomerular filtration rate (GFR) enhancing and natriuretic actions of NEP inhibition (25). Further, intrarenal administration of ANG II attenuated the actions of NEP inhibition. Originally, vasopeptidase inhibitors (VPIs) were developed to pursue the strategy of targeting NEP and RAAS. VPIs were single molecular entities with omapatrilat as the most advanced which inhibited NEP and ACE. This novel class possessed the renal and aldosterone suppressing actions of NEP inhibition together with the favorable hemodynamic actions of ACEI, and had lower risk of cardiovascular death or hospitalization (26). Their clinical development was stopped owing to the development of angioedema, however.

An alternative to the VPI strategy is the combination of NEP inhibition with angiotensin receptor blockade (27). Angiotensin receptor blockers (ARBs), unlike ACEI, do not alter

bradykinin metabolism that is thought to mediate the angioedema associated with VPIs. Thus, a new class of drugs has emerged which combines the actions of ARBs and NEP inhibition and this novel class is called angiotensin receptor-neprilysin inhibitors (ARNi's) (27).

LCZ696 (Entresto) is the most clinically advanced of the ARNi's and has recently been approved for the treatment of HF (27). LCZ696 is orally available and provides a 1:1 ratio blockade of AT1R in a valsartan moiety together with NEP inhibition with AHU377 (Sacubitril), a prodrug moiety, which is rapidly metabolized to an active moiety. In the landmark PARADIGM HF Trial reported by McMurray et al (n=8442), LCZ696 was compared with enalapril in patients who had HF with reduced ejection fraction (28). Because of an overwhelming benefit with LCZ696, the trial was stopped early. The seminal finding was that LCZ696 was superior to enalapril in reducing the risks of death and hospitalization for HF.

Designer NPs Resistant to NEP Degradation:

Native NPs include ANP, BNP and URO. Recombinant ANP (Carperitide) and BNP (Nesiritide) were approved for acute HF treatment. URO (Ularitide) is currently undergoing Phase III trial (TRUE AHF). Based on the biology of the NPs (Figure 1), the concept of designer NPs has emerged for the treatment of various cardiovascular, renal and metabolic diseases. Designer NPs are the result of novel peptide engineering in which strategic modifications in NP AA sequences are employed (29). Our rationale behind this concept is to produce chimeric NPs whose pharmacological and beneficial biological profiles go beyond those of the native NPs while minimizing undesirable effects. Another goal has been to engineer designer NPs that are highly resistant to NEP. The administration strategy of designer NPs in human studies is subcutaneous injection and it is expected they will be available as oral drugs in the future.

CD-NP (Cenderitide) is a novel 37 AA designer NP consisting of the mature 22 AA form of native human CNP fused with the 15 AA C- terminus of dendroaspis NP (DNP) (30). This first-generation designer NP retains the antifibrotic, antiproliferative, and antihypertrophic effects and venodilatation of CNP via pGC-B, as well as natriuretic and diuretic and aldosterone suppressing effects of DNP via pGC-A. Importantly, CD-NP has antiproliferative actions in cardiac fibroblasts and stimulates cGMP production in fibroblasts to a greater extent than BNP (30, 31). In vitro studies have demonstrated CD-NP is the first NP to activate both the pGC-A and the pGC-B receptor at physiological doses and is more resistant to proteolytic degradation than hANP, hBNP, and hCNP (4). In normal canines, intravenous infusion of CD-NP activates plasma cGMP and has natriuretic, diuretic, RAAS-suppressing actions, and unloads the heart with minimal effects on blood pressure (30). When compared to BNP (Nesiritide), CD-NP increased GFR and was less hypotensive than BNP. In a model of mild renal insufficiency and impaired diastolic function with cardiac fibrosis, chronic CD-NP prevented cardiac fibrosis and inhibited development of diastolic impairment (32). In healthy human subjects, CD-NP increased urinary and plasma cGMP concentrations, suppressed aldosterone, induced diuretic and natriuretic responses with a minimal reduction in mean arterial pressure (33). In March 2011, CD-NP received a fast-

track designation from the Food and Drug Administration and currently is in Phase II clinical trials targeting post acute HF patients using chronic subcutaneous infusion technology.

MANP (ZD100) is a best-in-class pGC-A activator designed at the Mayo Clinic, which consists of the 28 AA of ANP fused at the C-terminus to a novel 12 AA linear peptide (6). In vivo studies in normal canines and in models of hypertension and hypertensive HF, MANP is more natriuretic, cardiac unloading, aldosterone suppressing and blood pressure lowering than native ANP or nitroglycerin (6, 34, 35). The mechanism of these enhanced biological activities may be mediated by marked resistance to degradation by NEP. Like CD-NP, the elongated C-terminus may render MANP less susceptible to the actions of NEP (5). MANP has recently completed a Phase I Trial in humans with stable hypertension as well as in subjects with resistant hypertension. Specifically, Chen and co-workers reported that subcutaneous injection of MANP once daily reduced both systolic and diastolic blood pressure in patients with resistant hypertension for 24 hours. MANP increased GFR and sodium excretion while suppressing aldosterone (36). As there are no approved drugs for resistant hypertension and it is associated with increased risk for HF, stroke, myocardial infarction and chronic kidney disease, MANP may represent a therapeutic opportunity serving as a direct pGC-A activator but also being highly resistant to NEP degradation.

NEPRILYSIN AND IMPLICATIONS FOR BIOMARKERS IN HEART FAILURE

BNP, NT-proBNP as Biomarkers for HF:

BNP and the N-terminal proBNP (NT-proBNP) are widely used as gold standards for the diagnosis of acute HF (37, 38). Maisel and colleges reported that rapid measurement of BNP is useful in establishing or excluding the diagnosis of HF in patients with acute dyspnea (37). Further, NT-proBNP's diagnostic value for HF was established in a pool of 600 dyspneic patients presenting in the emergency department (38). BNP and NT-proBNP also have powerful prognostic value in HF patients, where baseline, predischarge values and changes have been shown to be associated with mortality and future outcomes (39).

As a preprohormone, pre-proBNP (134 AA) is processed to proBNP (108 AA), which is then cleaved by furin or corin to produce biologically active BNP (77–108, 32 AA) and the inactive fragment NTproBNP (1–76, 76 AA) (40) (Figure 2). BNP is a pGC-A activator and generates the second messenger cGMP in which pluripotent biological actions are induced. NT-proBNP is biologically inactive and does not bind to the pGC-A receptor. BNP's half life in humans is relatively short and BNP is rapidly degraded in human plasma through protease degradation and clearance receptor endocytosis. NT-proBNP is more stable and lasts longer in vivo than BNP. Evidence also suggests that there is a difference of BNP/NT-proBNP processing between acute and chronic HF. Vodovar and co-workers reported a difference with regard to the release of glycosylated proBNP (41, 42). In acute HF, increased release of proBNP involved release of more nonglycosylated proBNP which was rapidly processed to active BNP by furin. In contrast, increased production and release of proBNP in chronic HF involved release of more glycosylated proBNP, which is more resistant to enzymatic processing such as corin resulting in less biologically active circulating proBNP. Thus the diagnostic and prognostic value of BNP or proBNP may alter in the settings of

acute or chronic HF. One might speculate that with regard to BNP, a neprilysin inhibitor would be more effective in acute HF with increased concentrations of biologically active BNP as compared to chronic HF in which there is a high concentration of non-biologically active proBNP which has reduced pGC-A activity (43). This underscores again the importance of ANP as a substrate in chronic HF, recognizing the lack of glycosyation of proANP and the biologically active properties of both proANP and ANP upon pGC-A (44, 45).

"BNP Paradox" in HF:

As high values of plasma BNP have emerged as an effective biomarker for HF, its therapeutic benefits have been questioned, as increased BNP was perceived as not protecting against congestion, sodium and water retention and vasoconstriction, although chronic administration of BNP relieved HF symptoms in humans with HF. This "BNP Paradox" in HF was later partially explained by more in depth assays of BNP, NT-proBNP, and proBNP developed from mass spectrometry (MS) and specific monoclonal antibodies. Such work demonstrated that commercially available assays bind non-specifically to both proBNP, BNP and its degradation products, and most BNP-immunoreactive forms detected by immunoassay in HF represent proBNP. The results obtained from previous commercially available kits therefore do not necessarily represent the actual values of mature biologically active BNP (46-48). The work done by our laboratory (46) clearly demonstrated that in HF patients' plasma, either no BNP or very low BNP concentrations were detected by MS. Degradation products of BNP are rapidly formed in the plasma by proteases such as dipeptidyl peptidase IV and NEP during measurement. Higher proBNP values may be a result of (40) excessive production of proBNP by HF, impaired proBNP processing to BNP and/or accelerated BNP degradation. It should be noted however that unlike proANP which can activate pGC-A, proBNP is a poor activator of pGC-A which underscores that high concentrations of proBNP in HF represent a molecular form with reduced receptor activating properties.

As stated above, studies are consistent with the concept that NEP degrades ANP, BNP and NPs generate cGMP through the pGC-A receptor. Dual inhibition of NEP and angiotensin receptor blockade in HF patients also show increases in plasma BNP concentrations with reductions in NT-proBNP (49). Conventional theory is that increased BNP in HF patients indicates a worse prognosis and is a requirement for drug dose/regimen increases. However, increases in BNP with NEP inhibition are consistent with the drug effect on target. During NEP inhibition therapy, the substrates ANP, BNP are protected from degradation and thus their second messenger cGMP concentrations are increased as well. The increases in urinary cGMP (49) reflect the fact that the peptides' levels are enhanced by NEP inhibition acting through enhancement of cGMP. Further, the reduction in NT-proBNP, which is not degraded by NEP, is a signal for biological responsiveness to NPs/cGMP secondary to reductions in atrial pressures and NP secretion. Also, there is a hypothesis that Entresto may increase NT-proBNP and proBNP glycosylation, resulting in reduction of NT-proBNP and an increase of BNP measurements (50). The landmark work by Packer et al (49) supports the concept that BNP, NT-proBNP as well as cGMP values should be measured with NEP inhibition treatment to provide a thorough insight into HF pathophysiology and therapeutic action.

Predictive Value of Soluble NEP in HF:

Studies have documented an alternative processing form of NEP, soluble NEP, which exists in the plasma and urine (51, 52). Soluble NEP still possesses enzymatic activity to degrade peptides similarly as membrane bound NEP. In the experiments performed by Aviv et al (51), they were able to measure abundant amounts of NEP in the urine and its activity was dose-dependently inhibited by NEP inhibitors.

Soluble NEP has been reported recently to be associated with HF prognosis and outcomes. Specifically, circulating plasma NEP concentration have shown to be associated with future outcomes in chronic HF. Patients with higher NEP have significantly worse outcomes than those with a lower value in cardiovascular death or HF hospitalization (52). In another study in HF with preserved ejection fraction, investigators did not observe a significant association between soluble NEP concentrations and hospitalization for HF and/or death (53). The limited number of patients and trials supporting the positive association, and the inconsistency between these two studies raise the question of whether soluble NEP is a reliable biomarker for HF prognosis. Therefore plasma soluble NEP is a promising predictor for HF outcomes but its application in the clinic needs further investigation.

SUMMARY

NEP inhibition and NP therapeutics have grown as promising strategies for HF treatment. The success of LCZ696 (Entresto) supports the rationale that NEP inhibition with RAAS inhibition has more beneficial effects and reduces the risks caused by NEP inhibition alone. However, NEP inhibition generates off-target effects which warrant close surveillance. Another strategy, the use of NEP-resistant NPs, may be more specific. BNP and NT-proBNP are gold standard diagnostic biomarkers for HF. More and more evidence supports the conclusion that the “BNP paradox” is caused by inaccurate assay measurement in which plasma BNP is over-estimated by proBNP immunoreactivity (BNP deficiency, reduced BNP availability). Importantly, during ARNi treatment, BNP and cGMP were increased in parallel with a reduction of NT-proBNP, which supports the use of BNP, NT-proBNP and cGMP as a triad of biomarkers to be used with ARNi’s to guide treatment. Lastly, soluble NEP and its positive association with HF outcomes makes it a promising prognostic biomarker. However, its predictive value for HF is not well established and further investigations are needed.

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Nonstandard abbreviations:

NEP	neprilysin
NPs	natriuretic peptides
URO	urodilatin
AA	amino acid

DNP	dendroaspis NP
MANP	mutant atrial NP
ACEI	angiotensin converting enzyme inhibitor
VPIs	vasopeptidase inhibitors
ARB	angiotensin receptor blocker
ARNi's	angiotensin receptor-neprilysin inhibitors
CD-NP (Cenderitide)	a 37 AA designer NP consisting of the mature 22 AA form of native human C type NP fused with the 15 AA C-terminus of dendroaspis NP

REFERENCES

1. Kerr MA, Kenny AJ. The purification and specificity of a neutral endopeptidase from rabbit kidney brush border. Biochem J 1974;137:477–88. [PubMed: 4423492]
2. Turner AJ, Matsas R, Kenny AJ. Endopeptidase-24.11 and neuropeptide metabolism. Biochem Soc Trans 1985;13:39–42. [PubMed: 3888721]
3. Oefner C, D'Arcy A, Hennig M, Winkler FK, Dale GE. Structure of human neutral endopeptidase (neprilysin) complexed with phosphoramidon. J Mol Biol 2000;296:341–9. [PubMed: 10669592]
4. Dickey DM, Potter LR. Dendroaspis natriuretic peptide and the designer natriuretic peptide, cd-np, are resistant to proteolytic inactivation. J Mol Cell Cardiol 2011;51:67–71. [PubMed: 21459096]
5. Dickey DM, Yoder AR, Potter LR. A familial mutation renders atrial natriuretic peptide resistant to proteolytic degradation. J Biol Chem 2009;284:19196–202. [PubMed: 19458086]
6. McKie PM, Cataliotti A, Huntley BK, Martin FL, Olson TM, Burnett JC Jr. A human atrial natriuretic peptide gene mutation reveals a novel peptide with enhanced blood pressure-lowering, renal-enhancing, and aldosterone-suppressing actions. J Am Coll Cardiol 2009;54:1024–32. [PubMed: 19729120]
7. Kenny AJ, Bowes MA, Gee NS, Matsas R. Endopeptidase-24.11: A cell-surface enzyme for metabolizing regulatory peptides. Biochem Soc Trans 1985;13:293–5. [PubMed: 3894108]
8. Watanabe Y, Nakajima K, Shimamori Y, Fujimoto Y. Comparison of the hydrolysis of the three types of natriuretic peptides by human kidney neutral endopeptidase 24.11. Biochem Mol Med 1997;61:47–51. [PubMed: 9232196]
9. Kenny AJ, Bourne A, Ingram J. Hydrolysis of human and pig brain natriuretic peptides, urodilatin, c-type natriuretic peptide and some c-receptor ligands by endopeptidase-24.11. Biochem J 1993;291 (Pt 1):83–8. [PubMed: 8097089]
10. Sonnenberg JL, Sakane Y, Jeng AY, Koehn JA, Ansell JA, Wennogle LP, Ghai RD. Identification of protease 3.4.24.11 as the major atrial natriuretic factor degrading enzyme in the rat kidney. Peptides 1988;9:173–80. [PubMed: 2966343]
11. Walther T, Stepan H, Pankow K, Becker M, Schultheiss HP, Siems WE. Biochemical analysis of neutral endopeptidase activity reveals independent catabolism of atrial and brain natriuretic peptide. Biol Chem 2004;385:179–84. [PubMed: 15101560]
12. Condra CL, Leidy EA, Bunting P, Colton CD, Nutt RF, Rosenblatt M, Jacobs JW. Clearance and early hydrolysis of atrial natriuretic factor in vivo. Structural analysis of cleavage sites and design of an analogue that inhibits hormone cleavage. J Clin Invest 1988;81:1348–54. [PubMed: 2966813]
13. Smith MW, Espiner EA, Yandle TG, Charles CJ, Richards AM. Delayed metabolism of human brain natriuretic peptide reflects resistance to neutral endopeptidase. J Endocrinol 2000;167:239–46. [PubMed: 11054637]

14. Dickey DM, Potter LR. Human b-type natriuretic peptide is not degraded by meprin a. Biochem Pharmacol 2010;80:1007–11. [PubMed: 20599787]
15. Ozaki J, Shimizu H, Hashimoto Y, Itoh H, Nakao K, Inui K. Enzymatic inactivation of major circulating forms of atrial and brain natriuretic peptides. Eur J Pharmacol 1999;370:307–12. [PubMed: 10334507]
16. Pankow K, Wang Y, Gembardt F, Krause E, Sun X, Krause G, et al. Successive action of meprin a and neprilysin catabolizes b-type natriuretic peptide. Circ Res 2007;101:875–82. [PubMed: 17823376]
17. Abassi ZA, Golomb E, Agbaria R, Roller PP, Tate J, Keiser HR. Hydrolysis of iodine labelled urodilatin and anp by recombinant neutral endopeptidase ec. 3.4.24.11. Br J Pharmacol 1994;113:204–8. [PubMed: 7812611]
18. Martin FL, Stevens TL, Cataliotti A, Schirger JA, Borgeson DD, Redfield MM, et al. Natriuretic and antialdosterone actions of chronic oral nep inhibition during progressive congestive heart failure. Kidney Int 2005;67:1723–30. [PubMed: 15840018]
19. McDowell G, Nicholls DP. The endopeptidase inhibitor, candoxatril, and its therapeutic potential in the treatment of chronic cardiac failure in man. Expert Opin Investig Drugs 1999;8:79–84.
20. Vodovar N, Paquet C, Mebazaa A, Launay JM, Hugon J, Cohen-Solal A. Neprilysin, cardiovascular, and alzheimer's diseases: The therapeutic split? Eur Heart J 2015;36:902–5. [PubMed: 25636748]
21. Shipp MA, Tarr GE, Chen CY, Switzer SN, Hersh LB, Stein H, et al. Cd10/neutral endopeptidase 24.11 hydrolyzes bombesin-like peptides and regulates the growth of small cell carcinomas of the lung. Proc Natl Acad Sci U S A 1991;88:10662–6. [PubMed: 1660144]
22. Kuniyasu H, Luo Y, Fujii K, Sasahira T, Moriwaka Y, Tatsumoto N, et al. Cd10 enhances metastasis of colorectal cancer by abrogating the anti-tumoural effect of methionine-enkephalin in the liver. Gut 2010;59:348–56. [PubMed: 19828468]
23. Chen HH, Schirger JA, Chau WL, Jougasaki M, Lisy O, Redfield MM, et al. Renal response to acute neutral endopeptidase inhibition in mild and severe experimental heart failure. Circulation 1999;100:2443–8. [PubMed: 10595958]
24. Richards AM, Wittert G, Espiner EA, Yandle TG, Frampton C, Ikram H. Prolonged inhibition of endopeptidase 24.11 in normal man: Renal, endocrine and haemodynamic effects. J Hypertens 1991;9:955–62. [PubMed: 1658138]
25. Margulies KB, Perrella MA, McKinley LJ, Burnett JC Jr. Angiotensin inhibition potentiates the renal responses to neutral endopeptidase inhibition in dogs with congestive heart failure. J Clin Invest 1991;88:1636–42. [PubMed: 1658047]
26. Packer M, Califf RM, Konstam MA, Krum H, McMurray JJ, Rouleau JL, Swedberg K. Comparison of omapatrilat and enalapril in patients with chronic heart failure: The omapatrilat versus enalapril randomized trial of utility in reducing events (overture). Circulation 2002;106:920–6. [PubMed: 12186794]
27. von Lueder TG, Sangaralingham SJ, Wang BH, Kompa AR, Atar D, Burnett JC Jr., Krum H Renin-angiotensin blockade combined with natriuretic peptide system augmentation: Novel therapeutic concepts to combat heart failure. Circ Heart Fail 2013;6:594–605. [PubMed: 23694773]
28. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. N Engl J Med 2014;371:993–1004. [PubMed: 25176015]
29. Zakeri R, Burnett JC. Designer natriuretic peptides: A vision for the future of heart failure therapeutics. Can J Physiol Pharmacol 2011;89:593–601. [PubMed: 21815778]
30. Lisy O, Huntley BK, McCormick DJ, Kurlansky PA, Burnett JC, Jr. Design, synthesis, and actions of a novel chimeric natriuretic peptide: Cd-np. J Am Coll Cardiol 2008;52:60–8. [PubMed: 18582636]
31. Ichiki T, Schirger JA, Huntley BK, Brozovich FV, Maleszewski JJ, Sandberg SM, et al. Cardiac fibrosis in end-stage human heart failure and the cardiac natriuretic peptide guanylyl cyclase system: Regulation and therapeutic implications. J Mol Cell Cardiol 2014;75:199–205. [PubMed: 25117468]

32. Martin FL, Sangaralingham SJ, Huntley BK, McKie PM, Ichiki T, Chen HH, et al. Cd-np: A novel engineered dual guanylyl cyclase activator with anti-fibrotic actions in the heart. PLoS One 2012;7:e52422. [PubMed: 23272242]
33. Lee CY, Chen HH, Lisy O, Swan S, Cannon C, Lieu HD, Burnett JC Jr. Pharmacodynamics of a novel designer natriuretic peptide, cd-np, in a first-in-human clinical trial in healthy subjects. J Clin Pharmacol 2009;49:668–73. [PubMed: 19395584]
34. McKie PM, Cataliotti A, Boerrigter G, Chen HH, Sangaralingham SJ, Martin FL, et al. A novel atrial natriuretic peptide based therapeutic in experimental angiotensin ii mediated acute hypertension. Hypertension 2010;56:1152–9. [PubMed: 20975033]
35. McKie PM, Cataliotti A, Ichiki T, Sangaralingham SJ, Chen HH, Burnett JC Jr. M-atrial natriuretic peptide and nitroglycerin in a canine model of experimental acute hypertensive heart failure: Differential actions of 2 cgmp activating therapeutics. J Am Heart Assoc 2014;3:e000206. [PubMed: 24385449]
36. Chen HH, Neutel JM, Smith DH, Heublein D, Burnett JC. A first-in-human trial of a novel designer natriuretic peptide zd100 in human hypertension. J Am Soc Hypertens 2016;10:e23 (abstract).
37. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, et al. Rapid measurement of b-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–7. [PubMed: 12124404]
38. Januzzi JL Jr., Camargo CA, Anwaruddin S, Baggish AL, Chen AA, Krauser DG, et al. The n-terminal pro-bnp investigation of dyspnea in the emergency department (pride) study. Am J Cardiol 2005;95:948–54. [PubMed: 15820160]
39. Costello-Boerrigter LC, Burnett JC Jr. The prognostic value of n-terminal prob-type natriuretic peptide. Nat Clin Pract Cardiovasc Med 2005;2:194–201. [PubMed: 16265483]
40. Ichiki T, Huntley BK, Burnett JC Jr. Bnp molecular forms and processing by the cardiac serine protease corin. Adv Clin Chem 2013;61:1–31. [PubMed: 24015598]
41. Vodovar N, Seronde MF, Laribi S, Gayat E, Lassus J, Boukef R, et al. Post-translational modifications enhance nt-probnp and bnp production in acute decompensated heart failure. Eur Heart J 2014;35:3434–41. [PubMed: 25157115]
42. Ichiki T, Burnett JC Jr. Post-transcriptional modification of pro-bnp in heart failure: Is glycosylation and circulating furin key for cardiovascular homeostasis? Eur Heart J 2014;35:3001–3. [PubMed: 25246481]
43. Huntley BK, Sandberg SM, Heublein DM, Sangaralingham SJ, Burnett JC Jr., Ichiki T Pro-b-type natriuretic peptide-1–108 processing and degradation in human heart failure. Circ Heart Fail 2015;8:89–97. [PubMed: 25339504]
44. Jiang J, Pristera N, Wang W, Zhang X, Wu Q. Effect of sialylated o-glycans in pro-brain natriuretic peptide stability. Clin Chem 2010;56:959–66. [PubMed: 20348402]
45. Ichiki T, Huntley BK, Sangaralingham SJ, Burnett JC Jr. Pro-atrial natriuretic peptide: A novel guanylyl cyclase-a receptor activator that goes beyond atrial and b-type natriuretic peptides. JACC Heart Fail 2015;3:715–23. [PubMed: 26362447]
46. Hawkridge AM, Heublein DM, Bergen HR 3rd, Cataliotti A, Burnett JC Jr., Muddiman DC Quantitative mass spectral evidence for the absence of circulating brain natriuretic peptide (bnp-32) in severe human heart failure. Proc Natl Acad Sci U S A 2005;102:17442–7. [PubMed: 16293687]
47. Seferian KR, Tamm NN, Semenov AG, Mukharyamova KS, Tolstaya AA, Koshkina EV, et al. The brain natriuretic peptide (bnp) precursor is the major immunoreactive form of bnp in patients with heart failure. Clin Chem 2007;53:866–73. [PubMed: 17384012]
48. Liang F, O'Rear J, Schellenberger U, Tai L, Lasecki M, Schreiner GF, et al. Evidence for functional heterogeneity of circulating b-type natriuretic peptide. J Am Coll Cardiol 2007;49:1071–8. [PubMed: 17349887]
49. Packer M, McMurray JJ, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. Circulation 2015;131:54–61. [PubMed: 25403646]

50. Jaffe AS, Apple FS, Mebazaa A, Vodovar N. Unraveling n-terminal pro-b-type natriuretic peptide: Another piece to a very complex puzzle in heart failure patients. Clin Chem 2015;61:1016–8. [PubMed: 26078443]

51. Aviv R, Gurbanov K, Hoffman A, Blumberg S, Winaver J. Urinary neutral endopeptidase 24.11 activity: Modulation by chronic salt loading. Kidney Int 1995;47:855–60. [PubMed: 7752584]

52. Bayes-Genis A, Barallat J, Galan A, de Antonio M, Domingo M, Zamora E, et al. Soluble neprilysin is predictive of cardiovascular death and heart failure hospitalization in heart failure patients. J Am Coll Cardiol 2015;65:657–65. [PubMed: 25677426]

53. Goliasch G, Pavo N, Zotter-Tufaro C, Kammerlander A, Duca F, Mascherbauer J, Bonderman D. Soluble neprilysin does not correlate with outcome in heart failure with preserved ejection fraction. Eur J Heart Fail 2016;18:89–93. [PubMed: 26725876]

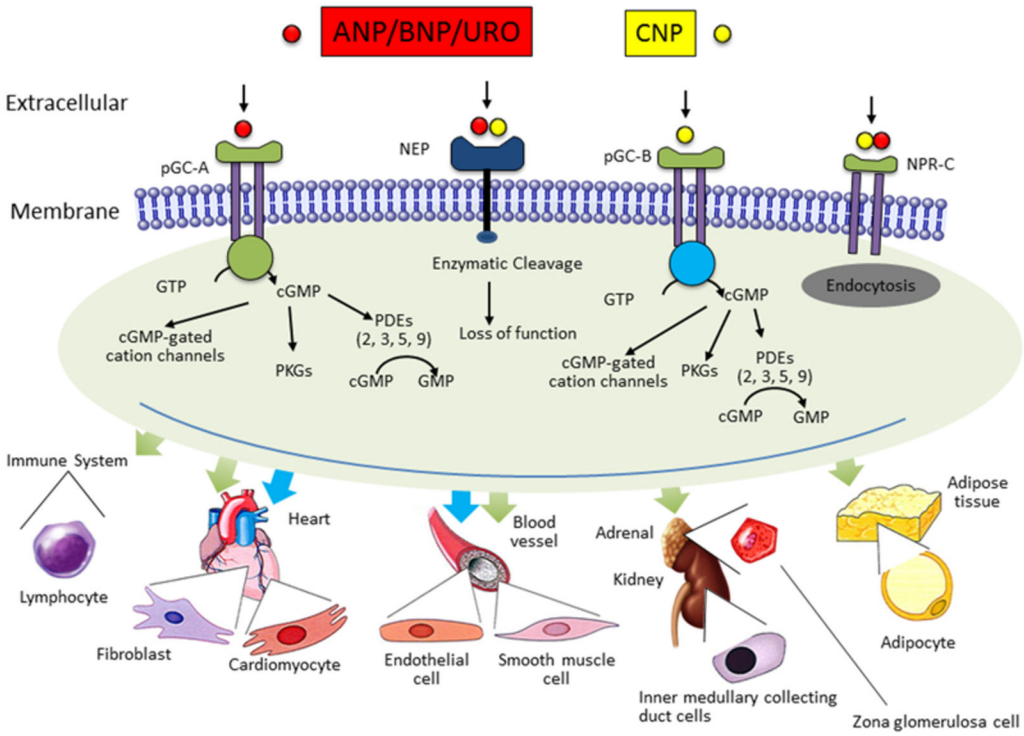

Figure 1. Natriuretic peptides signaling pathways and biological actions.

ANP/BNP/URO activate pGC-A and CNP activates pGC-B receptor, and all of which generate cGMP, which binds to protein kinase G (PKG), ion channels, and phosphodiesterases (PDEs). NP clearance receptor (NPRC) has no guanylyl cyclase activity and mediates NPs endocytosis. Neprilysin (NEP) is a major degrading enzyme. NPs induce pluripotent biological actions.

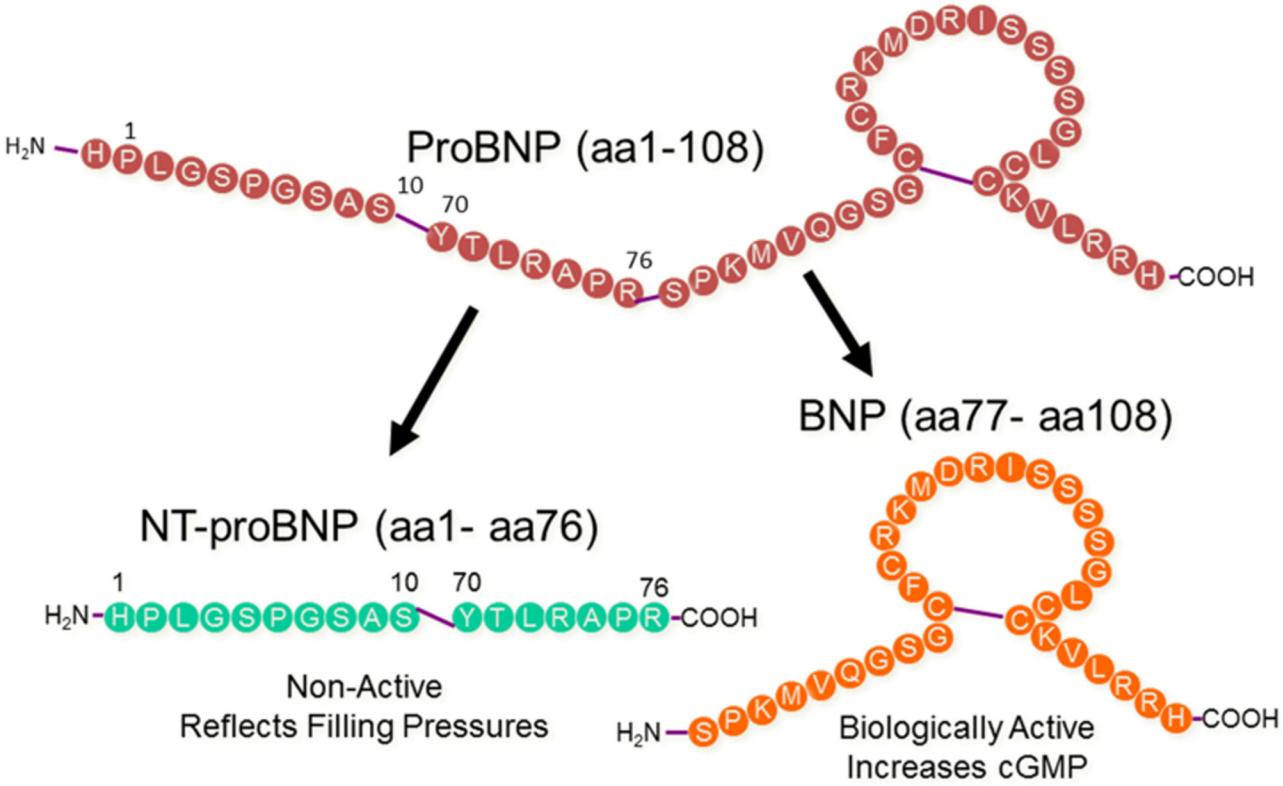

Figure 2. ProBNP processing to BNP and NT-proBNP.
ProBNP is processed by corin and furin to NT-proBNP (non-active) and mature BNP (biologically active via cGMP).

Table 1.

Neprilysin substrates and their biological actions, clinical relevance

Substrate	Biological actions of key substrates
Atrial natriuretic peptide	Induces natriuresis, diuresis, vasodilation, anti-fibrosis, and anti-RAAS.
B type natriuretic peptide	Induces natriuresis, diuresis, vasodilation, anti-fibrosis, and anti-RAAS. More resistant to NEP degradation than ANP or CNP.
Urodilatin	Induces enhanced renal effects with vasodilation, anti-fibrosis, and anti-RAAS. Less susceptible to NEP degradation compared to ANP or CNP.
C type natriuretic peptide	Induces vasodilation and anti-fibrosis. Highly susceptible to NEP degradation.
Enkephalin	Opioid receptor agonist, induces analgesia.
Substance P	Proinflammatory peptide, induces airway smooth muscle constriction.
Angiotensin II	Induces vasoconstriction.
Insulin B chain	Part of the insulin chains, controls blood sugar.
Endothelin	Vasoconstrictor.
Amyloid β	Substrate of Amyloid β polymer. Aβ degradation reduces the risk for Alzheimer's disease.
Bradykinin	Vasodilator, induces vasodilatation of epicardial coronary and resistance arteries in humans.
Bombesin-like peptides	Stimulate the growth of small cell carcinoma of the lung.