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MINIREVIEW

Natriuretic peptide metabolism, clearance and degradation

Lincoln R. Potter

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota-Twin Cities, Minneapolis, MN, USA

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Correspondence
L. R. Potter, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota-Twin Cities, 6–155 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, USA
Fax: +1 612 624 7282
Tel: +1 612 624 7251
E-mail: potter@umn.edu

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Atrial natriuretic peptide, B-type natriuretic peptide and C-type natriuretic peptide constitute a family of three structurally related, but genetically distinct, signaling molecules that regulate the cardiovascular, skeletal, nervous, reproductive and other systems by activating transmembrane guanylyl cyclases and elevating intracellular cGMP concentrations. This review broadly discusses the general characteristics of natriuretic peptides and their cognate signaling receptors, and then specifically discusses the tissue-specific metabolism of natriuretic peptides and their degradation by neprilysin, insulin-degrading enzyme, and natriuretic peptide receptor-C.

Introduction

General characteristics of natriuretic peptides and their receptors

All natriuretic peptides are synthesized as preprohormones that are processed to smaller mature forms containing an obligate C-terminal 17-residue disulfide ring structure. More detailed reviews of natriuretic peptides and their receptors are given in other articles in this series [1–3]. Atrial natriuretic peptide (ANP) is primarily stored as a propeptide in atrial granules, and is secreted and cleaved to a 28-residue mature peptide as it enters the circulation in response to atrial stretch. A version of ANP called urodilatin, containing four additional N-terminal residues, is primarily found in the kidney. B-type natriuretic peptide (BNP) is also present in atrial granules, but is found at the highest levels in ventricles from stressed hearts, such as those from congestive heart failure patients. BNP is not stored in granules in the ventricles. Instead, BNP is regulated at the transcriptional level. Plasma concentrations of ANP are several-fold higher than those of BNP in healthy humans [4,5]. Both ANP and BNP concentrations are elevated in patients with severe heart failure, and, in some cases, BNP levels exceed ANP levels [5–8]. Gene deletion experiments in mice indicate that ANP has broad systemic functions, lowering blood pressure and cardiac preload, whereas BNP primarily prevents fibrosis in the heart [9,10]. C-type natriuretic peptide (CNP) is found at low concentrations in the heart, and is present at higher

Abbreviations
ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; GC, guanylyl cyclase; IDE, insulin-degrading enzyme; NPRC, natriuretic peptide receptor-C.

concentrations in chondrocytes, where it stimulates long bone growth [11].

There are three known receptors for natriuretic peptides. Guanylyl cyclase (GC)-A is a particulate GC that catalyzes the synthesis of cGMP upon binding by ANP or BNP (Fig. 1) [12]. It contains a large extracellular ligand-binding domain, a single membrane-spanning region, and a large intracellular region composed of kinase homology domain regulatory, coiled-coil dimerization and GC catalytic domains. GC-B is homologous to GC-A, but is activated by CNP. Most physiological effects of natriuretic peptides are mediated by these two receptors. The best-characterized physiological functions associated with the activation of GC-A are renal sodium and water excretion, vasorelaxation, antagonism of the renin–angiotensin–aldosterone system, and endothelial extravasation [13]. In contrast, gene deletion studies in mice and familial mutations in humans provide compelling data indicating that the CNP–GC-B system mediates long bone growth [14–16].

All three natriuretic peptides also bind natriuretic peptide receptor-C (NPRC). NPRC is a disulfide-linked homodimer that is homologous to the extracellular domains of GC-A and GC-B, but contains only 37 intracellular amino acids (Fig. 1). It may also have signaling functions, but the majority of physiological data indicate that the primary role of NPRC is to clear natriuretic peptides from the extracellular environment via a receptor-mediated internalization and degradation process. NPRC binds all three family members with similar affinities. The half-life of [^{125}I]ANP in the circulation of NPRC null mice is two-thirds longer than in wild-type mice, although total ANP concentrations were not reduced in the null animals [17]. Additionally, mice lacking functional NPRC display mild hypotension, volume depletion and dilute urine associated with overactivation of GC-A, and elongated long bones and kyphosis associated with overactivation of GC-B [17,18]. In addition to undergoing receptor-mediated degradation, natriuretic peptides are also metabolized by extracellular proteases. Natriuretic peptide degradation is the focus of the remaining sections of this review.

Tissue-specific metabolism of natriuretic peptides

Natriuretic peptides are rapidly cleared from the body. Three mechanisms could formally contribute to this process: receptor-mediated degradation, degradation by extracellular proteases, and secretion of the peptides into body fluids such as urine or bile. As there is little evidence in support of the latter process under normal conditions [19], it will not be further considered here.

The ability of individual organs to remove molecules from the circulation is described by the extraction ratio, which is calculated by subtracting the venous concentration from the arterial concentration, and dividing this value by the arterial blood concentration of the molecule. This so-called *A/V* difference quantifies how efficiently the organ removes or degrades the molecule in question. The extraction ratio for ANP varies from about 20% to 75%, but is generally $\sim$ 35% for most organs [19]. To determine the net effect of the organ on whole body concentrations of

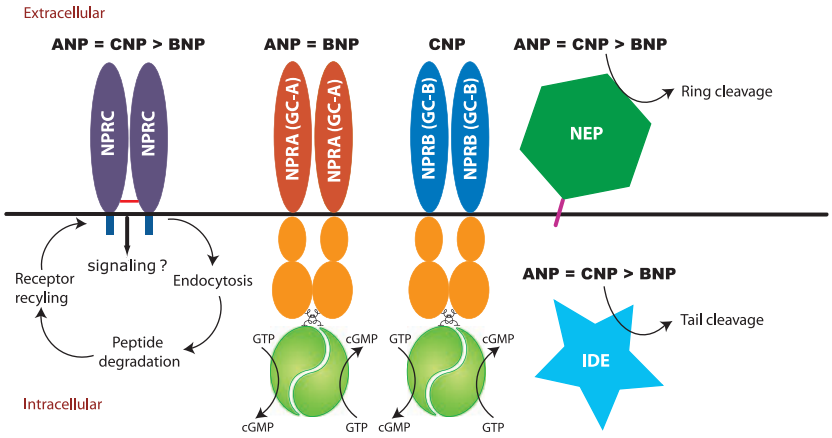

Fig. 1. Natriuretic peptides bind multiple cell surface proteins. Bold natriuretic peptide abbreviations indicate binding or substrate preference. NPRC internalizes all three natriuretic peptides, which targets them for degradation by intracellular proteases. Neprilysin is an extracellular metalloprotease that cleaves ANP and CNP at the Cys-Phe bond and breaks the ring. BNP is a much poorer substrate for neprilysin, and is not cleaved at the conserved Cys-Phe bond. IDE is depicted as a cytosolic enzyme - but it has also been found in membrane preparations [64]. It initially cleaves ANP and BNP outside the ring. NPRA, natriuretic peptide receptor-A; NPRB, natriuretic peptide receptor-B.

the molecule, organ blood flow also must be taken into consideration. Thus, the extraction ratio is multiplied by organ plasma flow to generate the organ clearance rate, which is described in units of liters per minute.

With these calculations, natriuretic peptide removal has been determined for several organs. However, caveats to the following discussion are that many of the human studies were conducted on sick patients, the antibodies used to detect ANP were generated against peptides smaller than the full-length 28-residue molecule, and blood sampling sites varied between studies. Additionally, the vast majority of reports evaluated ANP but not BNP or CNP.

With this information in mind, clearance of ANP can be generally characterized as relatively fast and on a par with the removal of other peptide hormones, such as vasopressin and angiotensin II. The reported half-life of ANP ranges from 0.5 to 4 min in mice, rats, rabbits, dogs, and monkeys [20], and is about 2 min in normal human subjects [21,22]. Most tissues remove ANP from the circulation, but some organs are more efficient at ANP extraction than others. Early human *A/V* studies indicated that about 30–50% of ANP is removed by the kidney, liver, or lower limbs, whereas no extraction was observed across the lung [23,24]. However, later reports in humans and dogs indicated that the lungs have a significant ANP extraction rate of between 19% and 24%. Importantly, lung shows the highest clearance of all organs (269 $mL \cdot min^{-1}$), owing to the high blood vessel surface area and perfusion rate of this tissue [25]. The difference between studies that observed significant versus no lung extraction appears to result from the use of different sampling sites [26]. The organ preference for ANP extraction is lung > liver > kidney [25].

Few studies have reported the clearance of BNP and CNP. Mukoyama *et al.* [5] originally observed that the removal of BNP from the human circulation is composed of short and long half-life components of 3.9 and 20.7 min, respectively. Other investigators reported a similarly long (22.6 min) half-life for BNP in humans [27]. Mukoyama *et al.* went on to report that BNP binds to human NPRC 7% as tightly as ANP, and suggested that the increased half-life of BNP results from decreased removal by NPRC-mediated internalization and degradation. The *A/V* differences of BNP are less than those observed for ANP in humans, consistent with the longer half-life of BNP [28].

CNP has the shortest half-life (2.6 min) of all of the natriuretic peptides in humans [29], and a similarly short half-life (1.6 min) in sheep [30]. When CNP was infused into sheep at rates of 1 or 10 $pmol \cdot kg^{-1}$ per min, metabolic clearance rates of 3.1 and 2.5 $L \cdot min^{-1}$, respectively, were observed. Like ANP, CNP is removed in dogs by the lungs, kidney, and vasculature of the lower body [31]. A recent study in humans reported positive CNP *A/V* gradients from the heart, head and neck, and musculoskeletal system, and negative gradients from renal, hepatic and pulmonary tissue, consistent with the former tissues secreting and the later tissues degrading CNP [32].

Receptor-mediated clearance of natriuretic peptides

NPRC-mediated ANP clearance was first demonstrated by Maack *et al.* in 1987 [33]. The key to these experiments was the development of C-ANF$^{4-23}$, an ANP analog missing the complete C-terminal tail as well as five amino acids within the disulfide ring. This analog preferentially binds NPRC over GC-A. With C-ANF$^{4-23}$ as a competing ligand, the vast majority of ANP-binding sites (> 90%) in the kidney and intact rat were attributed to NPRC. Perfusion of relatively high concentrations of C-ANF$^{4-23}$ (100 nM) into isolated kidneys did not stimulate the glomerular filtration rate or sodium excretion, consistent with the inability of this peptide to activate GC-A at the infused concentrations. However, infusion of C-ANF$^{4-23}$ into whole rats increased sodium excretion and decreased blood pressure in a manner that temporally correlated with elevations in the level of full-length ANP$^{1-28}$. Infusions of full-length ANP$^{1-28}$ to the levels observed during C-ANF$^{4-23}$ infusions yielded similar levels of sodium excretion and blood pressure reduction, consistent with C-ANF$^{4-23}$ blocking NPRC-mediated ANP degradation. C-ANF$^{4-23}$ infusions also markedly decreased the metabolic clearance, volume of distribution and appearance of radiolabeled hydrolytic products in anesthetized rats infused with [^{125}I]ANP [34]. A separate study reported that C-ANF$^{4-23}$ increased trichloroacetic acid-precipitable radiation from rats infused with [^{125}I]ANP by seven-fold, consistent with NPRC and/or other ANP-binding molecules playing a predominant role in mediated ANP degradation [35].

It is worth noting that the evolution of a separate receptor to clear peptide signaling molecules from the cardiovascular system is relatively unique to the natriuretic peptide system, because most other peptide signaling molecules, such as angiotensin II, endothelin, and vasopressin, are primarily degraded by extracellular proteases, and the vast majority of insulin is internalized by its cognate tyrosine kinase signaling receptor, not a separate non-tyrosine kinase receptor.

The cellular mechanics of NPRC-mediated natriuretic peptide internalization and degradation are

similar to those of the receptors for low-density lipoprotein, asialoglycoprotein, and hyaluronic acid. Similar features include lysosomal ligand hydrolysis and recycling of the ligand-free receptor back to the plasma membrane. Internalization is speculated to occur through a clathrin-dependent mechanism, but this has not been demonstrated. The effect of ligand binding on NPRC internalization is disputed, with one group indicating constitutive internalization and another group indicating downregulation [36,37]. The internalization rate of NPRC is about 5% per minute, and is inhibited by hyperosmotic sucrose, low temperature, and various agents that block lysosomal protein degradation [38]. Unlike GC-A, which rapidly releases ANP after binding [39], NPRC releases ANP more slowly than the rate of receptor internalization, ensuring that the majority of bound ligand is delivered to the lysosome for degradation. NPRC lacks known cytoplasmic internalization motifs such as NPXY and YXXZ (where X is any amino acid), which are common to other receptors that are internalized via a clathrin-coated pit-dependent pathway. Mutation of individual intracellular amino acids only marginally reduces the rate of NPRC internalization, but removal of the complete 37-residue intracellular domain decreases internalization by approximately 10-fold [38].

Proteolysis of natriuretic peptides

Natriuretic peptides are also degraded by extracellular proteases. Early studies indicated that rat and rabbit renal cortex brush border membranes, but not basolateral membranes, rapidly degrade human ANP [40,41]. Subsequent reports indicated that the inactivating cleavage occurs between Cys7 and Phe8 (Fig. 2), and is inhibited by the metal-chelating agents 1,10-phenanthroline and EDTA [42,43]. In pig microvillar membranes, ANP degradation was inhibited by phosphoramidon, an inhibitor of neprilysin (EC 3.4.24.11), which is also known as neutral endopeptidase, enkephalinase, common acute lymphoblastic leukemia antigen, and CD10 [44]. Neprilysin was initially discovered in rabbit kidney brush border membranes as a metalloenzyme that degrades the insulin β-chain [45], and subsequently as an enkephalinase [46] and β-amyloid-degrading enzyme. Neprilysin is a zinc-containing, membrane-bound, ectoenzyme that cleaves substrates on the amino side of hydrophobic residues [45] (Fig. 2). Stephenson *et al.* [47] demonstrated that the HPLC elution pattern of ANP cleavage products from kidney membranes was similar to that of the products produced when ANP was degraded by purified neprilysin. Subsequent studies indicated that ANP-degrading activity in solubilized rat membranes copurifies with neprilysin and is blocked by specific neprilysin inhibitors [48].

Purified neprilysin binds and degrades natriuretic peptides similarly to other peptide hormones such as angiotensin II [47]. Inhibition constants (K_i values) derived from blocking the degradation of the β-chain of insulin range from 2.5 μM for CNP to 172 μM for human BNP [49]. Seven ANP cleavage sites were identified (Arg4-Ser5, Cys7-Phe8, Arg11-Met12, Arg14-Ile15, Gly16-Ala17, Gly20-Leu21, and Ser25-Phe26), but the initial attack occurs between Cys7 and Phe8, breaking the ring and inactivating the peptide [47,50]. Initial neprilysin cleavage sites for ANP, BNP and CNP are shown in Fig. 2. Interestingly, a frameshift mutant of ANP containing 12 additional C-terminal amino acids is resistant to neprilysin degradation and is elevated in patients with familial atrial fibrillation [51,52]. Neprilysin also efficiently cleaves CNP at multiple sites (Cys6-Phe7, Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17, and Gly19-Leu20), and, as with ANP, the initial

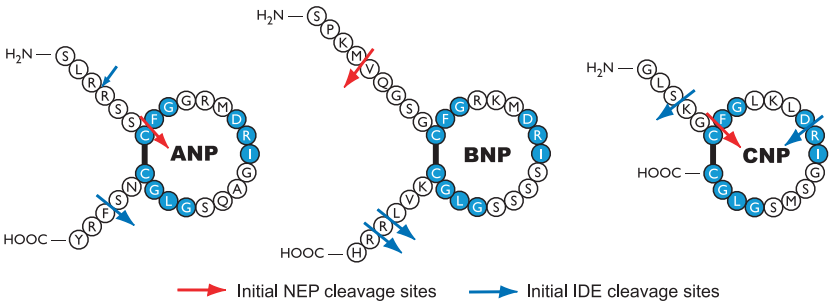

Fig. 2. Initial neprilysin (NEP) and IDE cleavage sites in human natriuretic peptides. Neprilysin data are from studies by Kenny *et al.*, Vanneste *et al.*, Norman *et al.* and Watanabe *et al.* [49,50,53,55]. IDE cleavage sites are from studies performed by Muller *et al.* with rat brain IDE and rat ANP, porcine BNP-26 and porcine CNP, and Ralat *et al.* with recombinant human IDE and human natriuretic peptides [68,69]. Large blue arrows indicate primary initial IDE sites. The small blue arrow indicates a minor initial IDE site in ANP. Both groups observed IDE cleavage of CNP between Asp12 and Arg13, but only Ralat *et al.* observed cleavage between Ser3 and Lys4.

cleavage site is between the conserved cysteine and phenyalanine residues [49,53]. The ring structures of both ANP and CNP are essential for hydrolysis, because reduction and alkylation of the peptides greatly reduced degradation [53].

In contrast to ANP or CNP, which have one or zero amino acid differences between human and rodent forms, BNP varies greatly between species [54]. For instance, rat BNP contains 45 residues and human BNP contains 32 residues, and there are 16 differences within the common 32-residue core structure. Studies with purified enzymes indicated that BNP is a poorer substrate for human or porcine neprilysin than ANP or CNP [49,53]. Neprilysin cleaves human BNP at Met5-Val6 and Arg17-Ile18, but not at the conserved Cys10-Phe11 bond [53,55]. Kenny *et al.* [49] found that cleavage at Met5-Val6 precedes cleavage at Arg17-Ile18 (Fig. 2). Urodilatin is a four-residue amino-extended form of ANP that is also a poorer substrate for neprilysin than ANP [56]. These data suggest that the additional terminal residues in ANP, BNP and urodilatin reduce access of neprilysin to the primary Cys10-Phe11 cleavage site [53]. Consistent with the idea of ANP being a better substrate for neprilysin, phosphoramidon dramatically increased ANP-dependent, but not BNP-dependent, cGMP elevation in mouse kidney slices [57]. The degradation preference of porcine neprilysin for human natriuretic peptides is CNP ≥ ANP > urodilatin >> BNP [49,53,56]. The k_{cat}/K_m values for human ANP, BNP and CNP are 5.1 $M^{-1}\cdot s^{-1}$, 0.5 $M^{-1}\cdot s^{-1}$, and 7.8 $M^{-1}\cdot s^{-1}$, respectively [53]. We recently demonstrated that neprilysin-dependent degradation of BNP is species-specific. Although neprilysin accounts for most of the BNP-degrading activity in rat kidney membranes, neprilysin inhibitors failed to block BNP degradation by human kidney membranes, suggesting that neprilysin is not a significant regulator of BNP concentrations in the human kidney [58].

Oral neprilysin inhibitors elevate natriuretic peptide concentrations in humans and animal models, and increase sodium excretion during heart failure, consistent with neprilysin or another enzyme that is blocked by neprilysin inhibitors contributing to natriuretic peptide degradation [56,59–62]. Natriuretic peptide levels have not been reported in mice lacking neprilysin, but these mice show no obvious signs of increased natriuretic peptide receptor activation, consistent with other degradation pathways compensating for the loss of neprilysin activity in this species [63].

ANP is also cleaved by insulin-degrading enzyme (IDE), a zinc metalloprotease that is found in both cytoplasmic and membrane fractions and has diverse substrate specificity (Fig. 1) [44,64]. Initial studies revealed that conditioned medium from smooth muscle and endothelial cells contained an EDTA-inhibited and EGTA-inhibited proteolytic activity that cleaves the bond between Ser25 and Phe26 of ANP [65,66]. Crosslinking studies by Muller *et al.* [67] revealed that [^{125}I]ANP binds with high affinity (K_d = 60 nM) to a cytosolic 112-kDa protein from rat olfactory bulb homogenates. On the basis of competition with insulin for ANP degradation and a partial amino acid sequence of the 112-kDa protein, IDE was suggested to be an ANP-degrading enzyme [67]. Additional studies demonstrated that ANP binding to IDE was blocked by full-length ANP but not by an ANP variant lacking the last three C-terminal residues or N-terminally truncated porcine BNP-26.

Proteolysis of rat ANP, porcine BNP-26 and CNP with purified IDE revealed that ANP is the preferred substrate [68]. The half-life for degradation of ANP by purified IDE was approximately one-third that for BNP or CNP. HPLC purification and MS analysis indicated that ANP was sequentially cleaved four times by IDE, whereas BNP and CNP were cleaved three and two times, respectively. ANP was initially cleaved at Ser25-Phe26 (Fig. 2), and then, in successive order and at much slower rates, at Arg3-Arg4, Asp13-Arg14, and Cys7-Phe8. Longer incubations with IDE resulted in the initial cleavage of BNP at Arg24-Arg25, followed by cleavage at Gly6-Arg7 and Asp10-Arg11. In contrast to initial cleavages outside the disulfide ring, CNP was initially cleaved between Asp12 and Arg13 (Fig. 2).

A recent and exciting report by Ralat *et al.* [69] suggests that IDE plays multiple roles in modulating the signaling response to natriuretic peptides. Like Muller *et al.*, they found that human IDE purified from *Escherichia coli* bound ANP five times more tightly (IC_{50} = 40 nM) than insulin. They also determined that human versions of ANP and CNP were much better IDE substrates than BNP, having K_{cat} values of 10 s^{-1}, 20 s^{-1}, and 0.2 s^{-1}, respectively.

Studies involving small interfering RNA knockdown of IDE in 293 cells stably expressing GC-A or GC-B revealed novel effects of IDE on receptor activation. Reduced IDE expression enhanced the stimulation of GC-A and GC-B by ANP and CNP, respectively, consistent with IDE-dependent degradation and inactivation of ANP and CNP. In contrast, reduced IDE expression was correlated with decreased activation of GC-A by BNP, consistent with IDE producing a superactive BNP variant. Incubation of these peptides with purified ICE increased and decreased the activation of GC-A by BNP and ANP, respectively.

Surprisingly, IDE exposure decreased the CNP activation of GC-B, but increased the cross-activation of GC-B by ANP and BNP. These *in vitro* data are consistent with IDE modulating natriuretic peptide potency and receptor preference.

Ralat *et al*. also determined major and minor cleavage sites of the natriuretic peptides (Fig. 2). Like Muller *et al*., they found that the major cleavage site of ANP was at Ser25-Phe26, but they also observed a small amount of cleavage products resulting from breaking the Arg3-Arg4 bond after incubating the peptide with IDE for 1 s. For this reason, they proposed a 'biased stochastic' as opposed to a 'sequential' cleavage model. Longer incubations resulted in near complete breakdown of ANP. The major cleavage sites for BNP were Leu29-Arg30 and Arg30-Arg31, whereas the major sites for CNP were Ser3-Lys4 and Phe12-Arg13. The general observation was that cleavage occurs first at the tails for peptides that have N-terminal and C-terminal extensions, but within the disulfide loop in peptides lacking extensions. As with neprilysin, the C-terminally extended frameshift mutant of ANP was a poor substrate for IDE. Interestingly, when IDE was incubated with mutant ANP and either wild-type ANP or CNP, the mutant peptide was preferentially and efficiently degraded. Finally, mice lacking IDE exhibit increased levels of amyloid β-protein and insulin, but natriuretic peptide concentrations in these animals have not been reported [70].

As described above, BNP is a poor substrate for neprilysin and IDE, suggesting that another protease is responsible for its cleavage. Consistent with this notion, Pankow *et al*. [71] reported that a 32-residue version of the normal 45-residue mouse BNP is degraded by the multimeric renal metalloprotease meprin A. Initial data indicated that BNP, but not ANP, is degraded similarly in wild-type and neprilysin 'knockout' mice, consistent with a neprilysin-independent proteolytic event. HPLC purification and MS identification showed that the initial meprin A cleavage site is at His6-Ile7. Interestingly, the resulting $BNP^{8–32}$ product retains the ability to activate GC-A in cell culture, but had reduced renal activating activity in dogs [71,72]. The His6-Ile7 sequence of the truncated mouse BNP used by Pankow *et al*. corresponds to Gln6-Gly7 in human BNP. Hence, the meprin A cleavage site is not conserved in human BNP and is not shown in Fig. 2. Protease inhibitor screening indicated that compounds known to inhibit meprin A (EDTA or actinonin) completely blocked BNP degradation in kidney membranes from the neprilysin-deficient animals. Purified mouse meprin A efficiently degraded mouse $BNP^{1–32}$, rat BNP, and porcine BNP, but not CNP. Importantly, $BNP^{1–32}$ degradation was severely reduced in kidney membranes from mice lacking meprin A, and cleavage of $BNP^{1–32}$ with meprin A increased the susceptibility of the peptide to ring cleavage by neprilysin. Thus, it was suggested that meprin A cleavage of $BNP^{1–32}$ facilitates subsequent cleavage and inactivation of $BNP^{7–32}$ by neprilysin [71]. However, the meprin A cleavage site is not conserved in human BNP, and we found that meprin A and neprilysin cleave rat BNP but do not cleave human BNP when measured in their respective kidney membrane preparations [58]. Interestingly, the serine protease inhibitor leupeptin was the most effective inhibitor of human BNP degradation, but the specific protease inhibited by leupeptin has not been identified.

Relative contributions of NPRC and neprilysin to natriuretic peptide degradation

The relative contributions of NPRC and neprilysin to ANP degradation have been investigated in a number of animal systems, with various NPRC-blocking peptides and neprilysin inhibitors. However, an assumption of these studies is that the NPRC-blocking peptides do not inhibit the proteases that degrade natriuretic peptides, and, to my knowledge, this has not been tested.

Under normal conditions, infusion of NPRC-blocking peptides has an effect on circulating ANP concentrations and associated physiological functions that is slightly greater than or equal to that of various neprilysin inhibitors [60,73–75]. However, in all cases examined, maximum ANP concentrations require inhibition of both degradation pathways. During pathological or pharmacological scenarios where natriuretic peptide concentrations are elevated and NPRC may be saturated, neprilysin plays a more significant role in ANP degradation [76]. Both NPRC and neprilysin pathways contribute to the degradation of BNP and CNP as well, although the exact contribution of each pathway to BNP concentrations is unclear [5,31,59,77]. In dogs, the total metabolic clearance rate of infused CNP was significantly reduced by infusion of $C\text{-}ANF^{4–23}$ or a neprilysin inhibitor [31,59]. Neprilysin inhibition reduced CNP clearance by the kidney but not the lung, suggesting that neprilysin significantly contributes to CNP degradation in some but not all tissues.

Conclusion and perspectives

A vast amount of data has been published regarding the metabolism and degradation of natriuretic peptides. From these reports, it is clear that NPRC is a

specific natriuretic peptide-degrading receptor, and that neprilysin and IDE are general proteases that degrade natriuretic and other peptides. However, recent reports of increased half-lives of natriuretic peptides associated with disease, as well as improved clinical benefits of proteolysis-resistant natriuretic peptides, suggest that natriuretic peptide degradation may be more important than previously appreciated [51,52,78,79].

Several important questions remain regarding natriuretic peptide degradation. Specifically, what role does IDE play *in vivo* in regulating natriuretic peptide concentrations, and does C-terminal cleavage by IDE produce natriuretic peptide variants with unique binding and activation characteristics? A specific IDE inhibitor would be extremely useful in illuminating the physiological significance of IDE in natriuretic peptide signaling. Other important questions are: what is the sequence of the BNP derivative produced by incubation with IDE, and what is the identity of the leupeptin-sensitive protease that degrades human BNP? Regarding receptor-dependent peptide clearance, several questions involving the molecular nature of NPRC internalization have yet to be answered. Although this receptor clearly internalizes and degrades natriuretic peptides, the molecular transport system and adaptor protein partners required for this process are not known. Finally, it remains to be seen whether ramifications of basic science research on natriuretic peptide degradation will find their way into the clinic. Can new versions of Nesiritide (recombinant human BNP), which is approved for the treatment of acute decompensated congestive heart failure, be engineered that are resistant to degradation or that have more desirable therapeutic profiles? For instance, can peptides be engineered that are degraded slowly in the kidney and rapidly in the vasculature? Only time will tell whether basic information on natriuretic peptide metabolism will translate into better therapeutic options for patients.

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