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Natriuretic Peptide Metabolism, Clearance and Degradation

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Abstract

Atrial natriuretic peptide, B-type natriuretic peptide and C-type natriuretic peptide compose 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, then specifically discusses the tissue specific metabolism of natriuretic peptides and their degradation by neprilysin, insulin-degrading enzyme and natriuretic peptide receptor-C.

Keywords

ANF; cardiovascular disease; endochondrial ossification; receptor internalization; signaling; proteolysis; ANP; BNP; CNP; cGMP; NEP

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-amino acid disulfide ring structure. Please see previous articles in this series for more detailed reviews of natriuretic peptides and their receptors [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 amino-terminal residues is primarily found in the kidney. B-type natriuretic peptide (BNP) is also in atrial granules, but is found at highest levels in ventricles from stressed hearts like 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 plasma concentrations 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 that lower 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 concentrations in chondrocytes where it stimulates long bone growth [11].

There are three known receptors for natriuretic peptides. Guanylyl cyclase-A (GC-A) is a particulate guanylyl cyclase that catalyzes the synthesis of cGMP upon binding by ANP or BNP (Fig. 1) [12]. It contains a large extracellular ligand-binding domain, single membrane-spanning region and a large intracellular region composed of kinase homology domain-regulatory, coiled-coil-dimerization and guanylyl cyclase-catalytic domains. Guanylyl cyclase-B (GC-B) is homologous to GC-A, but is activated by CNP. Most physiologic effects of natriuretic peptides are mediated by these two receptors. The best-characterized physiologic 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 to indicate that the CNP/GC-B system mediates long bone growth [14–16].

All three natriuretic peptides also bind natriuretic peptide receptor-C (NPR-C). NPR-C is a disulfide-linked homodimer that is homologous to the extracellular domains of GC-A and GC-B but only contains 37 intracellular amino acids (Fig. 1). It may also have signaling functions, but the majority of physiologic data indicate that the primary role for NPR-C is to clear natriuretic peptides from the extracellular environment via a receptor mediated internalization and degradation process. NPR-C binds all three family members with similar affinities. The half-life of ^{125}I-ANP in the circulation of NPR-C 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 NPR-C display mild hypotension, volume depletion and dilute urine associated with over-activation of GC-A and elongated long bones and kyphosis associated with over-activation of GC-B [17, 18]. In addition to 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 the body fluids like urine or bile. Since little evidence supports 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 around 35% for most organs [19]. To determine the net effect of the organ on whole body concentrations of 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.

Using 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, antibodies used to detect ANP were generated against peptides less than the full length 28 amino acid 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 par with removal of other peptide hormones like vasopressin and angiotensin II. The reported half-life of ANP ranges from 0.5 to 4 minutes in mice, rats, rabbits, dogs and monkeys [20] and is about two minutes 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 to 50% of ANP is removed by the kidney, liver or lower limbs, while 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 clearance is the highest of all organs (269 ml/min) due to the high blood vessel surface area and perfusion rate of this tissue [25]. The difference between studies that observed significant verses 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 and colleagues 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 minutes, respectively [5]. Other investigators reported a similarly long (22.6 min) half-life for BNP in humans [27]. Mukoyama and coworkers went on to report that BNP binds to human NPR-C 7% as tightly as it binds ANP and suggested that the increased half-life of BNP results from deceased removal by NPR-C-mediated internalization and degradation. 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 the natriuretic peptides in humans [29] and a similarly low half-life (1.6 min.) in sheep [30]. When CNP was infused in sheep at rates of 1 or 10 pmol/kg/min, metabolic clearance rates of 3.1 and 2.5 l/min, 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

NPR-C mediated ANP clearance was first demonstrated by Maack and colleagues in 1987 [33]. The key to these experiments was the development of C-ANF$^{4–23}$, an ANP analog missing the complete carboxyl-terminal tail as well as five amino acids within the disulfide ring. This analog preferentially binds NPR-C over GC-A. Using ANF$^{4–23}$ as a competing ligand the vast majority of ANP binding sites (> 90%) in the kidney and intact rat were

attributed to NPR-C. Perfusion of relatively high concentrations of C-ANF$^{4–23}$ (100 nM) into isolated kidneys did not stimulate 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 of full length ANP$^{1–28}$. Infusions of full length ANP$^{1–28}$ to levels observed during C-ANF$^{4–23}$ infusions, yielded similar levels of sodium excretions and blood pressure reductions, consistent with C-ANF$^{4–23}$ blocking NPR-C-mediated ANP degradation. C-ANF$^{4–23}$ infusions also markedly decreased 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 increased trichloroacetic acid-precipitable radiation from rats infused with ^{125}I-ANP by seven-fold, consistent with NPR-C 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 like angiotensin II, endothelin or vasopressin are primarily degraded by extracellular proteases, whereas 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 NPR-C mediated natriuretic peptide internalization and degradation is similar to that of the receptors for low-density lipoprotein, asialoglycoprotein and hyaluronic acid. Like 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 NPR-C internalization is disputed with one group indicating constitutive internalization and another group indicating downregulation [36, 37]. The internalization rate of NPR-C 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 that rapidly releases ANP after binding [39], dissociation of ANP from NPR-C is slower than the rate of receptor internalization, which ensures that the majority of bound ligand is delivered to the lysosome for degradation. NPR-C lacks known cytoplasmic internalization motifs like NPXY or YXXZ (where X is any amino acid) that 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 NPR-C internalization but removal of the complete 37 amino acid intracellular domain decreases internalization 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 C^{7} and F^{8} (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 (NEP) (EC 3.4.24.11), which is also known as neutral endopeptidase, enkephalinase, common acute lymphoblastic leukemia antigen and CD10 [44]. NEP was initially discovered in rabbit kidney brush border membranes as a metalloenzyme that degrades the insulin beta chain [45] and subsequently as an enkephalinase [46] and beta amyloid-degrading enzyme. NEP is a zinc-containing, membrane-bound, ectoenzyme that cleaves substrates on the amino side of hydrophobic residues [45] (Fig. 1). Stephenson and colleagues demonstrated that the HPLC elution pattern of ANP cleavage products from kidney membranes was similar to the products produced when ANP was degraded by purified NEP [47]. Subsequent studies indicated that ANP degrading activity in solubilized rat membranes copurifies with NEP and is blocked by specific NEP inhibitors [48].

Purified NEP binds and degrades natriuretic peptides similarly to other peptide hormones like angiotensin II [47]. Inhibition constants (Ki values) derived from blocking the degradation of the beta chain of insulin range from 2.5 μM for CNP to 172 μM for human BNP [49]. Seven ANP cleavage sites were identified (R^4-S^5, C^7-F^8, R^{11}-M^{12}, R^{14}-I^{15}, G^{16}-A^{17}, G^{20}-L^{21} and S^{25}-F^{26}) but the initial attack occurs between C^7 and $F^{8,}$ which breaks the ring and inactivates the peptide [47, 50]. Initial NEP cleavage sites for ANP, BNP and CNP are shown in Figure 2. Interestingly, a frameshift mutant of ANP containing 12 additional C-terminal amino acids is resistant to NEP degradation and is elevated in patients with familial atrial fibrillation [51, 52]. NEP also efficiently cleaves CNP at multiple sites (C^6-F^7, G^8-L^9, K^{10}-L^{11}, R^{13}-I^{14}, S^{16}-M^{17} and G^{19}-L^{20}), and like ANP, the initial cleavage site is between the conserved C and F residues [49, 53]. The ring structures of both ANP and CNP are essential for hydrolysis because reduction and alkylation of the peptides greatly decreases degradation [53].

In contrast to ANP or CNP, which have one or zero amino acid differences between human and rodent forms of the peptides, BNP varies greatly between species [54]. For instance rat BNP is 45-residues and human BNP is 32-residues with 16 differences within the common 32-residue core structure. Studies using purified enzymes indicated that BNP is a poorer substrate for human or porcine NEP compared to ANP or CNP [49, 53]. NEP cleaves human BNP at M^5-V^6 and R^{17}-I^{18} but not at the conserved C^{10}-F^{11} bond [53, 55]. Kenny and colleagues found that cleavage at M^5-V^6 precedes cleavage at R^{17}-I^{18} (Fig. 2)[49]. Urodilatin is a four residue amino-extended form of ANP that is also a poorer substrate for NEP than ANP [56]. These data suggest that the additional terminal residues in ANP, BNP and urodilatin reduce access of NEP to the primary C^{10}-F^{11} cleavage site [53]. Consistent with the idea of ANP being a better substrate for NEP, phosphoramidon dramatically increased ANP-dependent, but not BNP-dependent cGMP elevations, in mouse kidney slices [57]. The degradation preference of porcine NEP for human natriuretic peptides is CNP ≥ ANP > urodilation ≫ BNP [49, 53, 56]. The k_{cat}/K_m values for human ANP, BNP and CNP are 5.1 $M^{-1}s^{-1}$, 0.5 $M^{-1}s^{-1}$ and 7.8 $M^{-1}s^{-1}$, respectively [53]. We recently demonstrated that NEP-dependent degradation of BNP is species specific. Although NEP accounts for most of the BNP degrading activity in rat kidney membranes, NEP inhibitors failed to block BNP degradation by human kidney membranes, which suggest that NEP is not a significant regulator of BNP concentrations in the human kidney [58].

Oral NEP inhibitors elevate natriuretic peptide concentrations in humans and animals models and increase sodium excretion during heart failure, consistent with NEP or another enzyme that is blocked by NEP inhibitors contributing to normal natriuretic peptide degradation [56, 59–62]. Natriuretic peptide levels have not been reported in mice lacking NEP, but these mice show no obvious signs of increased natriuretic peptide receptor activation, consistent with other degradation pathways compensating for the loss of NEP 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- and EGTA-inhibited proteolytic activity that cleaves the bond between S^{25} and F^{26} of ANP [65, 66]. Cross-linking studies by Muller and colleagues revealed that ^{125}I-ANP binds with high affinity (Kd = 60 nM) to a cytosolic 112 kDa protein from rat olfactory bulb homogenates [67]. Based on competition with insulin for ANP degradation and partial amino acid sequence of the 112 kilodalton 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 amino-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 mass spectrometry 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 the S^{25}-F^{26} bond (Fig. 2), and then in successive order and at much slower rates, the R^{3}-R^{4}, D^{13}-R^{14} and C^{7}-F^{8} bonds were hydrolyzed. Longer incubations with IDE resulted in the initial cleavage of BNP at the R^{24}-R^{25} bond followed by cleavage at the G^{6}-R^{7} and D^{10}-R^{11} bonds. In contrast to initial cleavages outside the disulfide ring, CNP was initially cleaved between D^{12} and R^{13} (Fig. 2).

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

Studies involving siRNA 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 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 activation of GC-A by BNP and ANP, respectively. Surprisingly, IDE exposure decreased CNP activation of GC-B, but increased 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 and colleagues 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 the S^{25}-F^{26} bond, but they also observed a small amount of cleavage products from breaking the R^{3}-R^{4} bond after incubating the peptide with IDE for 1 sec. For this reason, they proposed a "biased stochastic" as opposed to "sequential" cleavage model. Longer incubations resulted in near complete breakdown of ANP. Major cleavage sites for BNP were between L^{29}-R^{30} and R^{30}-R^{31}, whereas major sites for CNP were between S^{3}-K^{4} and at the F^{12}-R^{13}. The general observation was that cleavage occurs first at the tails for peptides that have N- and C-terminal extensions, but within the disulfide loop in peptides lacking extensions. As with NEP, 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 beta protein and insulin, but natriuretic peptide concentrations in these animals have not been reported [70].

As described above, BNP is a poor substrate for NEP and IDE, which suggest that another protease is responsible for its cleavage. Consistent with this notion, Pankow and colleagues reported that a 32 amino acid version of the normal 45-residue mouse BNP molecule is degraded by the multimeric renal metalloprotease, meprin A [71]. Initial data indicated that BNP, but not ANP, is degraded similarly in wild type and NEP "knockout" mice, consistent with a NEP-independent proteolytic event. HPLC purification and mass spectrometric identification determined that the initial meprin cleavage site is at the H^{6}-I^{7} bond. Interestingly, the resulting $BNP^{8–32}$ product retains the ability to activate GC-A in cell culture but has reduced renal activating activity in dogs [71, 72]. The H^{6}-I^{7} sequence of the truncated mouse BNP used by Pankow and colleagues corresponds to Q^{6}-G^{7} in human BNP. Hence, the meprin 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 NEP deficient animals. Purified mouse meprin A efficiently degraded mouse BNP1-32, rat BNP and porcine BNP, but not CNP. Importantly, $BNP^{1–32}$ degradation was severely blunted in kidney membranes from mice lacking meprin A and cleavage of $BNP^{1–32}$ with meprin increased susceptibility of the peptide to ring cleavage by NEP. Thus, it was suggested that meprin A cleavage of $BNP^{1–32}$ facilitates subsequent cleavage and inactivation of $BNP^{7–32}$ by NEP [71]. However, the meprin cleavage site is not conserved in human BNP, and we found that meprin and NEP 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 contribution of NPR-C and NEP to natriuretic peptide degradation

The relative contribution of NPR-C and NEP to ANP degradation has been investigated in a number of animal systems using various NPR-C blocking peptides and NEP inhibitors.

However, an assumption of these studies is that the NPR-C blocking peptides do not inhibit the proteases that degrade natriuretic peptides, which has not been tested to my knowledge.

Under normal conditions, infusion of NPR-C blocking peptides has a slightly greater or an equal effect on circulating ANP concentrations and associated physiologic functions as various NEP 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 NPR-C may be saturated, NEP plays a more significant role in ANP degradation [76]. Both NPR-C and NEP 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, total metabolic clearance rate of infused CNP was significantly reduced by infusion of C-ANF$^{4–23}$ or a NEP inhibitor [31, 59]. NEP inhibition reduced CNP clearance by the kidney but not the lung, suggesting that NEP 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 NPR-C is a specific natriuretic peptide degrading receptor and that NEP 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 proteolytic resistant natriuretic peptides suggests 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 regulating *in vivo* natriuretic peptide concentrations, and does C-terminal cleavage by IDE produces natriuretic peptide variants with unique binding and activation characteristics? A specific IDE inhibitor would be extremely useful in illuminating the physiologic 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 NPR-C 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 its 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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Abbreviations

ANP	atrial natriuretic peptide
BNP	B-type natriuretic peptide
CNP	C-type natriuretic peptide
IDE	insulin degrading enzyme
NEP	neprilysin
GC-A	natriuretic peptide receptor-A
GC-B	natriuretic peptide receptor-B
NPR-C	natriuretic peptide receptor-C

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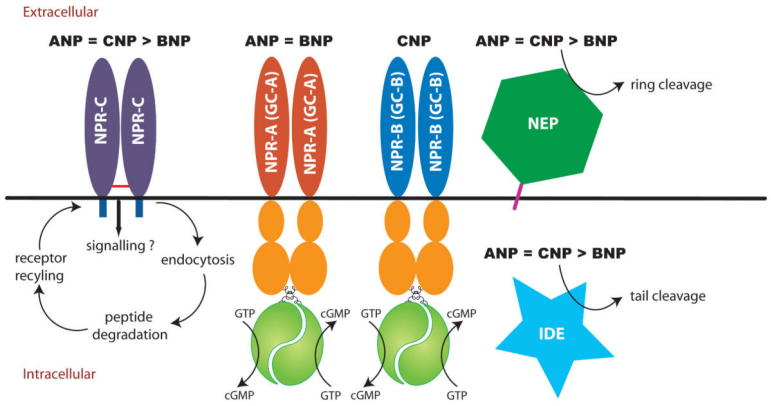

Figure 1.

Natriuretic peptides bind multiple cell surface proteins. Bolded black natriuretic peptide abbreviations indicate binding or substrate preference. NPR-C internalizes all three natriuretic peptides, which targets them for degradation by intracellular proteases. NEP is an extracellular metalloprotease that cleaves ANP and CNP at the C-F bond and breaks the ring. BNP is a much poorer substrate for NEP and is not cleaved at the conserved C-F bond. Insulin degrading enzyme (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.

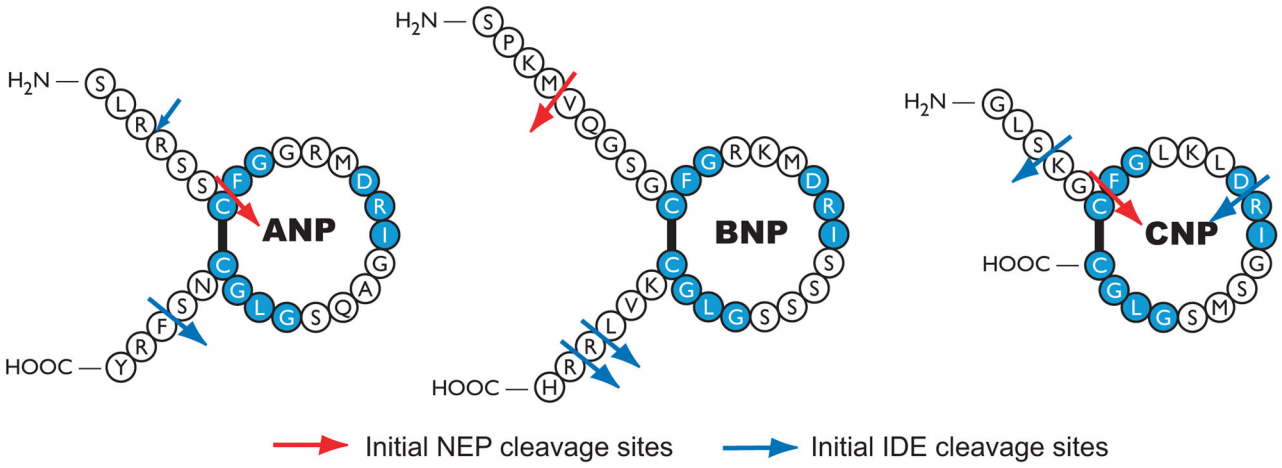

Figure 2.

Initial NEP and IDE cleavage sites in human natriuretic peptides. NEP 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 using rat brain IDE and rat ANP, porcine BNP-26 and porcine CNP and Ralat et al using recombinant human IDE and human natriuretic peptides [68, 69]. Large blue arrows indicates primary initial IDE sites. The small blue arrow indicates a minor initial IDE site in ANP. Both groups observed IDE cleavage of CNP between D^{12} and R^{13} but only Ralat et al observed cleavage between S^{3} and K^{4}.