Successive Action of Meprin A and Neprilysin Catabolizes B-Type Natriuretic Peptide

Kristin Pankow, Yong Wang, Florian Gembardt, Eberhard Krause, Xiaou Sun, Gerd Krause, Heinz-Peter Schultheiss, Wolf-Eberhard Siems, Thomas Walther

Abstract—Natriuretic peptides such as B-type natriuretic peptide (BNP) are important cardioprotective hormones with essential functions in sodium excretion, water balance and blood pressure regulation. Consequently, the catabolism of these peptides is in the focus of clinical research. In previous studies, we demonstrated that BNP, in contrast to the structurally related atrial and C-type natriuretic peptide, was not hydrolyzed by neprilysin (NEP). Because membrane preparations of several organs of NEP-knockout mice rapidly degrade BNP, the aim of this study was to identify BNP-catabolizing peptidases responsible for this fast clearance. Using kidney membranes of wild-type and NEP-knockout mice, as well as several peptidase inhibitors, we monitored the catabolism of BNP and analyzed its degradation products. We identified meprin A, a multimeric metalloprotease expressed in the brush borders of kidney proximal tubules, to initially truncate mouse BNP in the N terminus to mBNP7-32, a BNP metabolite with conserved biological activity. Consequently, in vivo experiments with the meprin inhibitor actoninin successfully elevated plasma BNP concentration in rats. We further demonstrated that the generation of mBNP7-32 is the prerequisite to catabolize BNP and identified NEP as the peptidase degrading the truncated BNP. Thus, the cooperative, successive action of the 2 transmembranal peptidases meprin A and NEP is crucial for rapid renal BNP inactivation. Therefore, the inhibition of meprin A could be a potent tool for increasing circulating BNP levels. (Circ Res. 2007;101:875-882.)

Key Words: B-type natriuretic peptide ■ natriuretic peptides ■ meprin ■ neutral endopeptidase ■ peptide degradation

Natriuretic peptides, as essential regulators of cardiovascular function in health and disease, are emerging as a focus of circulation research. Natriuretic peptides are naturally occurring cyclic peptide hormones with relevance to cardiovascular, endocrine, and renal homeostasis. They serve as functional antagonists of the renin–angiotensin system and cardiovascular, endocrine, and renal homeostasis. They serve as functional antagonists of the renin–angiotensin system and counteract fibrosis, cardiac hypertrophy, and remodeling in the heart.1–3 Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are secreted by cardiomyocytes in response to stretch or hormonal stimuli (angiotensin II, endothelin-1).2 C-Type natriuretic peptide (CNP) is mainly produced by endothelium and brain and acts as a local regulator of vascular tone and remodeling.4 The propeptides of the natriuretic peptides must be activated by limited proteolysis. Essentially involved in this activating process are serine peptidases, especially corin (S1 family) for ANP and furin (S8 family) for BNP and CNP.5–7 All 3 natriuretic peptides share a highly conserved 17-aa ring structure formed by a Cys–Cys disulfide bridge. This intact ring system is essential for biological activity.8 ANP and BNP mediate their functions via the natriuretic peptide receptor A (NPRA) and CNP via the natriuretic peptide receptor B (NPRB); both receptors are linked to a guanylyl cyclase, which leads to the production of the second messenger cGMP. Natriuretic peptides are rapidly removed from the circulation by extracellular peptidases or by the natriuretic peptide clearance receptor.2 All activating and degrading processes are critical for the regulation of natriuretic peptide activity. Consequently, the benefits of the natriuretic peptide system could be used in 4 different ways: (1) by activation of the natriuretic peptide-converting enzymes corin and furin, (2) by intravenous injection of recombinant natriuretic peptides, (3) by administration of natriuretic peptide clearance receptor antagonists, or (4) by inhibition of natriuretic peptide-catabolizing enzymes.

The latter, the inhibition of natriuretic peptide catabolism, obligates a detailed knowledge of degrading peptidases. Because an intact ring structure is crucial for biological activity of natriuretic peptides,8 total inactivation requires a cleavage within the ring. For ANP and CNP, such an inactivating ring opening has been described through the peptidolytic attack by neprilysin (NEP) (EC 3.4.24.11; also
named enkephalinase or neutral endopeptidase) cleaving the Cys–Phe bond within the ring near the disulfide bridge.9,10 For BNP, this inactivation remains debatable. Our own previous studies and the results of other groups clearly demonstrate a resistance of BNP toward NEP.11,12 Thus, we intended to identify the peptidase(s) responsible for the clearance of BNP and therefore to determine a new target for pharmacological intervention in the treatment and prevention of cardiovascular diseases.

Materials and Methods

Materials
The NEP inhibitor candoxatrilat was a gift from Pfizer (Karsruhe, Germany). Actinomycin was a gift from Solvay (Hanover, Germany). Mouse BNP 1-32 (mBNP) and mouse BNP 7-32 (mBNP7-32) were synthesized by Biosyntan (Berlin, Germany); rat BNP 1-32 (tBNP) were synthesized by AnaSpec (San Jose, Calif); mouse ANP (mANP) and the rat BNP ELA kit were purchased from Bachem (Weil am Rhein, Germany); and CNN and porcine BNP 1-32 (pBNP) were from Phoenix Europe (Karlsruhe, Germany). Recombinant mouse NEP, meprin α and β subunits, and the CgMP kit were from R&D Systems (Wiesbaden, Germany). The protease inhibitor set was purchased from Genotech (St Louis, Mo), and the PolyFect reagent was from Qiagen GmbH (Hilden, Germany). NPRA was purchased from Origen Technologies (Rockville, Md). NPRB was a gift provided generously by Michael Bader (MDC, Berlin, Germany). Cell culture products were purchased from Invitrogen (Karsruhe, Germany). All other chemicals were from Sigma (Taufkirchen, Germany). CD rats were purchased from Charles River (Sulzfeld, Germany).

Membrane Preparation
Kidney membranes of NEP-knockout mice and their wild-type controls, both on the same pure genetic background (C57BL/6)13,14 and other mouse strains (Balb/c, C3H/HeJ, CBA/J), were prepared according to the method of Hulme and Buckley.15 In brief, whole kidneys were homogenized at 4°C in 50 mmol/L Tris buffer (pH 7.4) by a glass–Teflon potter, then filtered through nylon gauze and centrifuged at 40,000g to separate membranes from the cytosol.

After washing, the final pellet was resuspended in the 5-fold volume of 50 mmol/L Tris buffer containing 1% Triton X-100 and 0.1% sodium dodecyl sulfate for solubilization. The remaining suspension was stored at –80°C. The total protein contents of the membrane preparations were determined according to the method of Bradford16 and adjusted to 5.3 mg/mL or 0.4 mg/mL depending on the experiment.

Degradation of mANP and mBNP by Mouse Kidney Membrane

mANP or mBNP (10 μmol/L) were incubated with 80 μL of solubilized kidney membrane of wild-type and NEP-knockout mice (0.4 mg/mL protein) in a final volume of 600 μL of Tris buffer supplemented with 0.1% BSA (Tris/BSA) in presence of the angiotensin-converting enzyme inhibitor bestatin (100 μmol/L) and additional candoxatrilat (0.1 mmol/L) for NEP-independent degradation. Samples of 100 μL were repeatedly taken during an incubation time of 120 minutes and stopped with 50 μL of 0.35 mol/L perchloric acid. Samples were analyzed by high-performance liquid chromatography (HPLC).

Mass Spectrometry

Degradation products were manually collected after HPLC separation and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Measurements were performed on a Voyager-DE STR BioSpectrometry Workstation (PerSeptive Biosystems, Framingham, Mass). HPLC samples (1 μL) were mixed with 1 μL of α-cyano-4-hydroxycinnamic acid solution consisting of 10 mg of matrix dissolved in 1 mL of 0.3% trifluoroacetic acid in acetonitrile-water (1:1; vol/vol). From the resulting mixture, 1 μL was applied to the sample plate. Samples were air dried at ambient temperature (24°C) and measured in the reflectron mode at an acceleration voltage of 20 kV. Each spectrum obtained was the mean of 250 laser shots. To identify peptide fragments that resulted from cleavage of BNP from their experimental masses, the program FindPept (http://www.expasy.ch/tools/findpept.html) was used. The monoisotopic masses were considered, and the mass tolerance was set to 0.2 Da.

Use of Inhibitors

Five samples, each of 10 μmol/L mBNP, were incubated with 5 μL of solubilized kidney membrane of NEP-knockout mice (5.3 mg/mL protein) in a final volume of 100 μL of Tris/BSA in the presence of effective concentrations of different peptidase inhibitors. Reactions were stopped after 30 minutes with 50 μL of 0.35 mol/L perchloric acid. Most of the inhibitors were part of a protease inhibitor set that contained ready-to-use individual protease inhibitors in 100-fold effective concentration. Lisinopril was used in a final concentration of 20 μmol/L and actinomycin in a final concentration of 1 μmol/L. In the experiment with candoxatrilat/actinomycin, 3 samples of 10 μmol/L mBNP were incubated with 5 μL of solubilized wild-type kidney membrane (5.3 mg/mL protein) at 37°C in a final volume of 100 μL of Tris/BSA in the presence of 100 μmol/L bestatin or additionally with 100 μmol/L candoxatrilat and/or 1 μmol/L actinomycin and stopped after 20 minutes. Degradation of mBNP and formation of mBNP7-32 were monitored by HPLC.

Degradation of Natriuretic Peptides by Meprin Subunits

Meprin subunit α or β was activated with trypsin according to the protocol (R&D Systems, Wiesbaden, Germany). Natriuretic peptide (10 μmol/L) was incubated with 0.3 mg/mL meprin α or β subunit at 37°C in a final volume of 300 μL of Tris/BSA. Samples of 80 μL were taken after 0, 15, and 30 minutes, stopped with 40 μL of 0.35 mol/L perchloric acid and analyzed by HPLC.

Strain-Specific Differences in Renal Meprin Activity

mBNP (10 μmol/L) was incubated at 37°C with 12 μL of kidney membrane of different mouse strains (2 animals per strain) (0.4 mg/mL protein) in a final volume of 90 μL of Tris buffer supplemented with 0.1% BSA (Tris/BSA) in the presence of the angiotensin-converting enzyme inhibitor bestatin (100 μmol/L) and candoxatrilat (0.01 mmol/L). Samples (n = 3 per kidney, 2 animals per strain) were stopped with 45 μL of 0.35 mol/L perchloric acid after 30 or 60 minutes, respectively. Samples were analyzed by HPLC.

Degradation of Natriuretic Peptides by NEP

Natriuretic peptide (10 μmol/L) was incubated with 0.27 μg/mL recombinant mouse NEP in a final volume of 900 μL of Tris/BSA. Samples were taken in triplicate after 0, 20, and 60 minutes, stopped with 0.35 mol/L perchloric acid, and analyzed by HPLC. In presence of 0.1 mmol/L of the NEP inhibitor candoxatrilat, NEP activity was completely suppressed (data not shown).

High-Performance Liquid Chromatography

All samples were centrifuged at 12 000g and analyzed by a HPLC system (Shimadzu LC10-AD, Kyoto, Japan) with a Nucleosil C18 column (100-Å pore size, 5-μm particle size, 250 × 4.6 mm; VDS optilab, Berlin, Germany), UV detection at 216 nm, 50-μL injection volume, and a flow rate of 1 mL/min. A linear correlation between peak areas and natriuretic peptide concentration was confirmed before analysis. mANP and mBNP and its degradation products were eluted within 25 minutes using a linear gradient of 20% to 32% acetonitrile for mANP and 20% to 35% for mBNP in 0.1% trifluoroacetic acid, pBNP and CNP and its degradation products were stored at 50 mmol/L Tris buffer containing 1% Triton X-100 and 0.1% acetonitrile-water (1:1; vol/vol) for mANP or mBNP (10 μmol/L) were incubated with 80 μL of solubilized kidney membrane of wild-type and NEP-knockout mice (0.4 mg/mL protein) in a final volume of 600 μL of Tris buffer supplemented with 0.1% BSA (Tris/BSA) in presence of the angiotensin-converting enzyme inhibitor bestatin (100 μmol/L) and additional candoxatrilat (0.1 mmol/L) for NEP-independent degradation. Samples of 100 μL were repeatedly taken during an incubation time of 120 minutes and stopped with 50 μL of 0.35 mol/L perchloric acid. Samples were analyzed by high-performance liquid chromatography (HPLC).
were eluted within 15 minutes using a linear gradient of 25% to 35% acetonitrile in 0.1% trifluoroacetic acid.

cGMP Measurements
Cultured aortic smooth muscle cells (SMCs) were derived from the explants of the thoracic aortas of 4- to 6-week old male C57BL/6 mice and were grown in DMEM supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. SMCs at the fourth to fifth passage were used. Human embryonic kidney cells (HEK293) were cultured in DMEM supplemented with 10% FBS and penicillin–streptomycin (100 IU/mL to 100 μg/mL). The cultures were maintained at 37°C in a 5% CO2 humidified incubator. One day before transfection, HEK293 cells were plated in 24-well cell culture dishes and then transiently transfected with 500 ng of DNA every well at 80% confluence using PolyFect reagent. Transfection was performed in DMEM with 0.5% FBS without antibiotics. Nontransfected cells served as controls. After 24 hours, cells were treated with vehicle, 1 μmol/L mBNP, 1 μmol/L CNP, or 1 μmol/L mBNP7-32 for 5 minutes. After washing 3 times in cold Hank’s balanced salt solution, cells were resuspended in 150 μL of cell lysis buffer provided in a cGMP kit. cGMP concentrations were measured according to the instructions of the manufacturer and expressed as picomoles per milliliter. The same protocol was applied to aortic SMCs, except transfection.

Bioactivity In Vivo
Male CD rats (200 to 250 g body weight) received actinonin (100 mg/kg body weight) or an equal volume of solvent intraperitoneally twice within 24 hours. Directly before the second injection and 12 hours later, blood was taken under isoflurane anesthesia (24 and 36 hours after the first injection) by puncture of the retroorbital venous plexus. Blood samples were put on ice for 30 minutes to clot and then centrifuged (4°C; 10 minutes; 3000 rpm). Serum was snap-frozen in liquid nitrogen and stored at −80°C until further processing. Serum BNP levels were measured using a commercial kit according to the instructions of the manufacturer.

Statistics
For statistical comparisons, Student’s t test was used (means±SEM). Significance was considered at a value of P<0.05.
tion mixture and possible intermediate products mBNP2-32 up to BNP6-32 were not detected.

Screening for Peptidase(s) Initially Degrading BNP
To further characterize the peptidase that initially truncates mBNP in kidney membrane, we tested a broad spectrum of group-specific peptidase inhibitors (Figure 2A, white columns). The mBNP-degrading activity was completely inhibited by the metallopeptidase inhibitor EDTA. Minor effects were found for other inhibitors such as chymostatin. Consequently, respecting the abovementioned allocation of the enzyme as endopeptidase, the main BNP-degrading activity should come from an enzyme belonging to the group of metalloendopeptidases (EC 3.4.24).

Notably, phosphoramidon, which inhibits an important part of metalloendopeptidases, eg, NEP, NEP2, and endothelin-converting enzyme, had no significant effect. This rapid constriction of eligible enzymes enabled us in a next step to prove specific metallopeptidase inhibitors (Figure 2A, hatched columns). Described metallopeptidases in kidney membranes are several amino- and carboxypeptidases. Whereas successive aminopeptidolytical action could be excluded with the aminopeptidase inhibitor bestatin (Figure 2A), a carboxypeptidase effect was also implausible because we observed an endopeptidolytic cleavage site on the N-terminal tail of the cyclic mBNP structure. We could also exclude NEP, NEP2, and endothelin-converting enzyme because of the phosphoramidon insensitivity of BNP degradation.

Further metallopeptidases located on kidney membrane are angiotensin-converting enzyme, an enzyme that acts sometimes as an endopeptidase, and the enzymes of the meprin group that are involved in the degradation of a variety of peptides, including bradykinin, neurotensin, or luteinizing hormone-releasing hormone. The most specific angiotensin-converting enzyme inhibitor lisinopril had no inhibitory effect on mBNP degradation. However, the meprin inhibitor actinonin blocked the conversion of mBNP to mBNP7-32 (Figure 2A). Thus, our strategy to identify the responsible enzyme stepwise describes the peptidase in question as an endopeptidase, a phosphoramidon-insensitive metalloendopeptidase, and finally as meprin. Additionally, the inhibitory profile of our BNP degradation resembles meprin properties: Kenny and Ingram described the insensitivity of meprin to phosphoramidon and Yamaguchi et al. detected that its activity was inhibited by EDTA but also slightly by chymostatin. This completely corresponds to our mBNP degradation results in the presence of group-specific peptidase inhibitors (Figure 2A, white columns).

Figure 2. Screening for peptidase(s) degrading BNP. A, Degradation of mBNP by kidney membrane of NEP-knockout mice in the presence of effective concentrations of peptidase inhibitors; values are shown as means±SEM (n=5). B, Degradation of natriuretic peptides by recombinant meprin (n=3; SD within the points). C, Comparison of HPLC chromatograms of mBNP during incubation with meprin α or mouse kidney membrane with or without the meprin inhibitor actinonin (peak 1: mBNP; peak 2: mBNP7-32). D, mBNP degradation by kidney membrane of mouse strains lacking meprin α expression (filled symbols, C3H/HeN and CBA/J) and normal meprin α expression (open symbols, Balb/c and C57BL/6 wild-type and NEP-knockout [k.o.] mice); 2 animals per strain (n=3 per kidney); SD within the points.
Meprin A Initially Degrades BNP

Meprin proteases consist of evolutionary-related subunits called meprin α and β. Because there are clear differences in the substrate preferences of the α and β subunits, we tested the mBNP degradation with the corresponding recombinant proteins. The meprin α subunit degraded mBNP in a time-dependent manner. However, CNP, which is the best substrate for NEP among the 3 natriuretic peptides, was not degraded by the meprin α subunit (Figure 2B). We also investigated BNP from 2 other species, rBNP and pBNP. Both BNs were degraded by the meprin α subunit with the same rate as mBNP. Importantly, the meprin β subunit did not degrade mBNP (data not shown).

Meprins containing α subunits are called meprin A (EC 3.4.24.18). Meprin A exists either as a homooligomer consisting of only meprin α subunits or as a heterooligomer consisting of meprin α and β subunits. Because the α subunit is cleaved from the membrane during maturation, membrane-bound meprin A also requires the β subunit. Consequently, we conclude that the kidney membrane-bound BNP-degrading activity comes from the heterooligomeric meprin A, because the α subunit was required for activity and the β subunit was required for membrane-bound localization.

This has been further supported by comparing the HPLC chromatograms of mBNP metabolites after treatment with meprin α or kidney membrane (Figure 2C). In both experiments, mBNP degradation led to the same major product (peak 2 at 28.3 minutes), which was identified as mBNP7-32 by mass spectrometry before (Figure 1C). Actinonin, a meprin inhibitor, completely inhibited the formation of this product.

In experiments using pBNP (Figure 2B), we also observed the N-terminal truncated form pBNP7-32 as the primary metabolite, confirmed with a control peptide by HPLC (data not shown). Notably, this pBNP7-32 is identical to the so-called pBNP26 found by Sudoh et al in porcine brain. Finally, we identified rBNP8-32 (m/z 2783 with intact disulfide bridge) and rBNP7-32 (m/z 2696 with intact disulfide bridge) as the main products of rBNP degradation by meprin α (data not shown).

Meprin A is a multimeric metalloendopeptidase that is expressed mainly in mammalian kidney and intestine. Stephenson and Kenny already described ANP degradation by meprin A, but, when comparing the prominent ANP degradation by NEP, meprin was not the dominant ANP-degrading enzyme. Because BNP degradation by meprin had not yet been tested, we demonstrate, for the first time, that meprin A initiates specifically the catabolism of BNP in murine kidney.

Impaired mBNP Degradation in Kidneys of Mouse Strains Lacking Meprin α Expression

Beynon and Bond reported about mouse strains that lack meprin α expression and therefore meprin A activity. We used this information in a control experiment comparing mBNP degradation in kidney membranes of these non–meprin α–expressing strains (C3H/HeN and CBA/J) with the degradation in our C57BL/6 wild-type and NEP-knockout mice, both characterized by normal meprin α expression, and in an additional control strain (Balb/c). Degradation of mBNP in the strains C3H/HeN and CBA/J was significantly delayed compared with the meprin α–expressing strains Balb/c and C57BL/6 (Figure 2D; P<0.001). This strongly supports our primary data, because it proves that meprin A is indeed the peptidase responsible for the observed mBNP degradation in kidney membranes.

mBNP7-32 Keeps Biological Actions

The initial degradation product of mBNP in kidney membrane, mBNP7-32, has a truncated N terminus but still contains the intact ring structure. To test the bioactivity of the truncated product and to compare the possible activity with equal concentrations of mANP, mBNP, and CNP, we used cultured mouse aortic SMCs that endogenously express both NPRA and NPRB receptors. However, mBNP7-32 also has been able to stimulate cGMP generation, and, when comparing its potency with that of mature mBNP, there was no significant difference but a tendency toward decreased activity. Thus, the intermediate is still biologically active.

To determine whether the N-terminal truncation of mBNP decreases its affinity toward the NPRA or even shifts it toward NPRB, we tested cGMP production in selectively NPRA- or NPRB-transfected HEK293 cells (Figure 3B and 3C). Both mBNP and mBNP7-32 induced cGMP production in the NPRA-transfected cells, but mBNP was more potent than mBNP7-32 (Figure 3B), demonstrating that the truncation reduces but preserves the bioactivity of BNP. The NPRB ligand CNP, as a negative control, had no influence on cGMP production in NPRA-transfected cells. In NPRB-transfected cells, neither mBNP nor mBNP7-32 induced cGMP production, and thus an N-terminal truncation of mBNP does not shift the receptor affinity toward NPRB. This underlines that the length of the N-terminal tail of the different natriuretic peptides is not decisive for the described receptor selectivity of ANP/BNP and CNP. Because CNP lacks the C-terminal tail, and mBNP7-32 has only a shorter N-terminal tail, the missing C terminus seems to be crucial for the specific binding of CNP to NPRB.

mBNP7-32 Is Catabolized by NEP

As shown in figure 3A, mBNP7-32 is still active, but with its shorter N-terminal tail, it becomes structurally more similar to ANP. We therefore investigated whether mBNP7-32 may become a better substrate for NEP as the full-length mBNP. To test this, we compared the degradation of mANP, mBNP, and mBNP7-32 by recombinant mouse NEP (Figure 4A). Whereas mBNP was extremely slowly degraded by NEP, the degradation of mBNP7-32 was comparable to the rapid degradation of mANP, demonstrating NEP to be critically involved in the final inactivation of mBNP.
To further clarify this, we performed mBNP degradation studies with murine wild-type kidney membranes pretreated with the NEP inhibitor candoxatrilat, the meprin inhibitor actinonin, or both inhibitors together (Figure 4B and 4C) and observed mBNP degradation and mBNP7-32 formation.

Actinonin was most efficient in inhibiting mBNP degradation, whereas candoxatrilat did not reveal a significant effect compared with nontreated membranes, confirming meprin A as the initial peptidase catalyzing mBNP. Notably, in the actinonin-treated membranes, mBNP7-32 formation was almost prevented, and thus meprin A is the only peptidase converting mBNP to mBNP7-32 in kidney membranes. On the other hand, mBNP7-32 was significantly enriched in the candoxatrilat-treated membranes ($P<0.01$), demonstrating that NEP decisively participates in the second step of mBNP degradation, cleaving and thus inactivating the metabolite mBNP7-32.

Taken together, we observed for the first time a successive action of 2 membrane-bound kidney peptidases to catalyze a biologically important peptide: meprin A truncates mBNP under
formation of mBNP7-32. This metabolite is still active but is now accessible for inactivation by NEP. Thus, the truncation by meprin A is the prerequisite for fast mBNP clearance.

In Vivo Accumulation of BNP by Actinonin

Because meprin α, a subunit of meprin A, is the initially BNP-degrading enzyme, it becomes important as a potential target for pharmacological intervention. The inhibition of meprin α may potentiate BNP action by peptide accumulation because it disables BNP truncation and therefore delays BNP inactivation by NEP. In an in vivo experiment, we treated rats with the meprin inhibitor actinonin (100 mg/kg body weight) and measured plasma BNP levels after 24 and 36 hours. Compared with the control group (solvent), the actinonin-treated animals significantly accumulated circulating BNP (Figure 5). This strongly underscores our in vitro data and proves the in vivo relevance of meprin α for BNP catabolism. Notably, because actinonin has been discussed also to inhibit the activity of the secreted matrix metalloproteinase 2 and 9,36 we tested for the ability of these matrix metalloproteinases to degrade BNP and could exclude it (data not shown). Consequently, meprin α inhibition is an effective approach to elevate BNP levels and thus may become a promising tool for therapeutic use.

In contrast to ANP, mBNP is not initially degraded by NEP. The reason for this lacking degradation is probably the bigger size of the N-terminal tail. Therefore, the truncation of this tail allows quick BNP catabolism by NEP. Meprin α generates the metabolite mBNP7-32 that still binds to NPRA, thus stimulating cGMP production, and is the target for NEP degradation (Figure 6).

There are several trials that utilize the beneficial effects of natriuretic peptides in hypertension and heart failure. For example, intravenous application of synthetic human BNP was found to improve hemodynamic function in patients with decompensated congestive heart failure.37 Other approaches focus on NEP inhibitory compounds. NEP inhibitors are actually developed to prevent natriuretic peptide degradation and consequently to enhance their biological activity. However, in clinical trials, NEP inhibitors did not show the expected blood pressure effects38 because vasoconstrictors such as angiotensin II and endothelin-1 are also NEP substrates and consequently accumulate during treatment with a NEP inhibitor. To circumvent this effect, dual angiotensin-converting enzyme/NEP inhibitors were recently developed to combine the hypotensive actions of angiotensin-converting enzyme inhibition by preventing angiotensin II generation with the inhibition of natriuretic peptide degradation. The negative side effects of these so-called “vasopeptidase inhibitors,” such as higher rates of angioedemas,39,40 require careful examination. Furthermore, new results of basic research generally question the usage of NEP-inhibiting compounds. Recently, NEP was described as a key enzyme in the catabolism of amyloid-β, the so-called “Alzheimer-peptide.” Consequently, NEP inhibitors or the mentioned mixed inhibitors are able to disturb the delicate steady state of anabolic and catabolic processes of amyloid-β.41 Although most NEP inhibitors probably do not cross the blood–brain barrier, this amyloid-β argument may prospectively prevent any long-term NEP inhibitor use in clinical practice.

Here, we present meprin α, a peptidase that initiates BNP degradation. Meprin α could be a potential target to therapeutically increase cardioprotective BNP. We thus identified a new approach for BNP-directed pharmacological interventions and point to a new direction of research to potentiate BNP actions.

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Disclosures

None.

References


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