Meprin A metalloprotease and its role in acute kidney injury

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Submitted 9 January 2013; accepted in final form 13 February 2013

Meprin A metalloprotease and its role in acute kidney injury. Am J Physiol Renal Physiol 304: F1150–F1158, 2013. First published February 20, 2013; doi:10.1152/ajprenal.00014.2013.—Meprin A, composed of α- and β-subunits, is a membrane-associated neutral metalloendopeptidase that belongs to the astacin family of zinc endopeptidases. It was first discovered as an azocasein and benzoyl-L-tyrosyl-p-aminobenzoic acid hydrolase in the brush-border membranes of proximal tubules and intestines. Meprin isoforms are now found to be widely distributed in various organs (kidney, intestines, leukocytes, skin, bladder, and a variety of cancer cells) and are capable of hydrolyzing and processing a large number of substrates, including extracellular matrix proteins, cytokines, adhesion junction proteins, hormones, bioactive peptides, and cell surface proteins. The ability of meprin A to cleave various substrates sheds new light on the functional properties of this enzyme, including matrix remodeling, inflammation, and cell-cell and cell-matrix processes. Following ischemia-reperfusion (IR)- and cisplatin-induced acute kidney injury (AKI), meprin A is redistributed toward the basolateral plasma membrane, and the cleaved form of meprin A is excreted in the urine. These studies suggest that altered localization and shedding of meprin A in places other than the apical membranes may be deleterious in vivo in acute tubular injury. These studies also provide new insight into the importance of a sheddase involved in the release of membrane-associated meprin A under pathological conditions. Meprin A is injurious to the kidney during AKI, as meprin A-knockout mice and meprin inhibition provide protective roles and improve renal function. Meprin A, therefore, plays an important role in AKI and potentially is a unique target for therapeutic intervention during AKI.

acute kidney injury; meprin; metalloprotease; renal proximal tubule

Meprins and Identification of Their Expression in the Kidney and Other Tissues

Meprins are zinc-dependent metalloproteinasises of the astacin family that were initially isolated and characterized from brush-border membranes of the mouse (10) and rat (48) kidneys and human intestines (74). Meprins are abundantly expressed at the apical membranes of the proximal tubules in the corticomedullary junction and comprise ~5% of the total proteins of kidney brush-border membranes (12, 21). Meprin expression has also been identified in human skin epithelia (5), brain (44), leukocytes in the lamina propria of the human inflamed bowel (58), and mesenteric lymph nodes (22), colorectal carcinoma (59), breast cancer cells (61), primary ovarian mucinous carcinoma and gastrointestinal cancers (32), and in glomeruli of some strains of rat (65). In addition, expression of meprins has also been reported in salivary glands, craniofacial death of proximal tubular epithelial cells by apoptosis and necrosis, leading to denuded areas of tubular epithelium and exposing the ECM to degradation. Recruitment of leukocytes and associated inflammation are also important mediators of AKI. This review highlights that meprin plays important roles in many of these events.

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regions during development, ureter, and bladder (20, 72) and documented by EST analyses in the human spleen, pancreas, and liver (MEROPS peptidase database, http://merops.sanger.ac.uk; EMBL-EBI ArrayExpress Warehouse, http://www.ebi.ac.uk/arrayexpress). In nonmammals, the occurrence of meprins has been reported, so far, in the intestinal epithelium, epidermis, and lamina propria mucosae of zebrafish (Danio rerio) (69, 70). Meprin expression in brush-border membranes of the kidney and intestines and its expression pattern in the skin epidermis suggest that meprin expression is unique to the highly differentiated epithelium.

Most of the original work on the structure and oligomeric assembly of meprins has been performed by Bond and colleagues (13, 76). Meprins are expressed as oligomeric forms of closely related meprin α- and/or meprin β-subunits (Fig. 1), with 42% identity in amino acid sequences between the subunits (4, 7, 29, 41). When expressed as a single isoform independently, meprin α- or meprin β-subunits form homodimers by formation of disulfide bridges. Meprin β-homodimers are membrane-associated, whereas meprin α-homodimers are not membrane bound but can self-associate further to become higher molecular mass oligomers, which becomes the largest sized secreted protease (4, 7, 40). Meprin α- and meprin β-isoforms, when coexpressed, form heterodimers by disulfide linkage and may further associate by homophilic interactions to create heterotetramer structures (Fig. 1) (7, 60). The homo-oligomeric form of α-subunits and hetero-oligomeric form of α and β are known as meprin A while the homo-oligomeric form of the β-subunit is known as meprin B (Fig. 1). Meprin A, comprising α- and β-subunits, is the major form and is expressed abundantly in the apical membranes of the renal proximal tubules as an integral membrane protein (12, 21, 47, 53). Meprin A is anchored to the brush-border membranes through the membrane-spanning domain of the β-subunit (7, 60, 82). Both meprin α- and β-subunits are initially synthesized as proteins containing the transmembrane domains but, during biosynthesis, the membrane-spanning domain of the α-subunit is proteolytically cleaved (30, 79). The β-subunit with its intact transmembrane domain is transported and anchored to the brush-border membrane as a type 1 integral plasma membrane protein. The mature form of meprin α is retained by the β-subunit by disulfide linkages or is secreted as a large homooligomer into the extracellular space. Thus meprin A, containing both α- and β-subunits, is a membrane-associated protease anchored to the brush-border membranes only through the β-subunit.

### Structural Domains of Meprin A

Meprin subunits are type-I membrane proteins composed of multiple domains (Fig. 1). The domain structure comprises a signal peptide; propeptide; catalytic domain containing the zinc-binding active site motif HExxHxGxxH/N joined by a unique conserved methionine-containing β-hairpin, Met turn (77); a meprin A5 protein tyrosine phosphatase μ (MAM) domain, a meprin-and-TRAF (tumor necrosis factor receptor-associated factor) homology (MATH) domain; an intervening domain, also called AM (after MATH) domain; an epidermal growth factor (EGF)-like domain; a C-terminal transmembrane domain; and a cytosolic domain (76). In addition, the α-subunit has an I (inserted)-domain after the AM domain. The N-terminal propeptide, including the signal peptide, comprises 62–66 amino acids in both subunits (29) and must be cleaved for the activation of the meprins. The zinc-binding motif and a Met turn form a deep cleft for substrate binding (77).

The MAM domain in meprins, also found in many unrelated membrane proteins, is involved in adhesion function (6) and is known to facilitate protein-protein interactions (1). Cysteine residues in the MAM domains are involved in the formation of disulfide bonds that contribute to the production of oligomeric structures in meprins (41). Like the MAM domain, the TRAF domain is also present in many intracellular proteins and participates in protein-protein interactions (36, 90) and plays a role in a disulfide bridge formed between the meprin subunits (41). Thus both the MAM and TRAF domains participate in the oligomerization of meprins. The specific function of the AM domain is unknown; however, it is speculated that this domain may provide an extension from the membrane for the meprin molecule to participate in the catalytic function (81). Although the nucleotide sequence of both meprin α and meprin β genes contain sequences for C-terminal EGF, transmembrane, and cytosolic domains, only the mature form of β contain these domains but they are proteolytically removed.
from the mature α-subunit (28, 60, 62). The C-terminal transmembrane and cytoplasmic domain of the β-subunit is involved not only in membrane insertion but also in intracellular transport (30, 57). The cytosolic domain of meprin β comprising 28 amino acids has a phosphorylation site for protein kinase C and calmodulin kinase (31). This domain plays a role in its export from the endoplasmic reticulum to the Golgi apparatus (57).

**Cell- and Tissue-Specific Expression and Secretion of Meprin Subunits**

Meprin subunits are expressed together or independently, and their expression and secretion are tissue and cell dependent. In the apical brush-border membranes of proximal tubules and intestinal epithelium, both meprin subunits are co-expressed, but cultured renal proximal tubular epithelial cells or intestinal epithelial cells do not synthesize the meprin isoforms. Also, primary cells prepared from proximal tubules do not express meprins, probably because of the loss of brush-border membranes. Colon cancer Caco2 cells constitutively express meprin α (but not meprin β) and secrete it from both apical and basolateral plasma membranes of the cells (59), suggesting that this secretion may increase the potential of colon tumor cells to invade the ECM. On the other hand, kidney proximal tubules and intestines secrete meprin α homooligomers only from the apical surface of into the lumen. Polarized Madin-Darby canine kidney (MDCK) cells cotransfected with both meprin α and β are sorted to the apical surface; both human meprin α and β are secreted whereas transfected mouse meprin β is not secreted (25). In the meprin β-transfected MDCK cells, secretion of meprin β was constitutive as well as regulated by protein kinase C. In this study it was proposed that ADAM-17 was responsible for the ectodomain shedding of meprin-β (31). However, a recent study has demonstrated that ADAM-10 is the major enzyme for ectodomain shedding of meprin β in stably cotransfected human embryonic kidney (HEK) cells with human meprin α and β (Herzog C, Haun RS, Ludwig A, Shah SV, Kauschal GP, unpublished observations). However, whether ADAM-10 is involved in shedding of meprin A in vivo during AKI remains to be determined. After it has been verified to be a sheddase of meprin A in vivo during AKI, it will be of interest to further examine the role of ADAM-10 in AKI using pharmacological or genetic approaches. In the human epidermis, meprin α and meprin β are constitutively expressed in separate layers of the skin (5). Expression patterns of meprin subunits in different layers of the epidermis suggest distinct functions of each subunit in keratinocyte differentiation and proliferation. The expression of meprin subunits in the mouse kidney is also dependent on the strain of mouse. All of the strains of mouse examined, including C57BL/6, C3H/He, CBA, and ICR, express both subunits of meprin during fetal stages, but after birth, C3H/He and ICR strains downregulate meprin α expression and are thus deficient in meprin A (53). This trait in the kidney is inherited as an autosomal recessive trait (11). Meprin A is undetected in the urine of meprin α-deficient strains (21). The other strains expressing both subunits normally excrete homomeric meprin A in the urine (9, 21). In zebrafish, two different α-subunits and one β-subunit are encoded by three meprin genes present on chromosome 20 (69, 70).

**Immunolocalization of Meprin A and Its Subunits in the Kidney**

Immunohistochemical localization studies using polyclonal antibodies to the meprin A purified from kidney cortices revealed a specific linear staining pattern in the brush-border of normal proximal tubules in the corticomedullary junction (3, 21, 35, 84). Meprin A staining in mouse kidneys was exclusively observed in the brush-border membranes of the proximal tubules and it was undetected in the luminal surface of distal tubules, glomeruli, and collecting ducts (3, 21, 35, 84). Since membrane-associated meprin A is composed of α- and β-subunits, it is essential to examine whether both subunits show colocalization in the apical membranes of proximal tubules using subunit-specific antibodies. Localization of meprin-β with a meprin-β-specific antibody was similar to that observed with a meprin A antibody (15, 35). Our recent studies have demonstrated colocalization of both meprin α and β in the apical membranes of mouse proximal tubules using subunit-specific antibodies (Fig. 2A). These antibodies did not stain the luminal surface of distal tubules, glomeruli, or collecting ducts. These studies further support the notion that meprin A, composed of α- and β-subunits, is restricted exclusively to the brush-border membranes.

**Properties and Activation of Meprin A**

Meprin A is not inhibited by classic matrix metalloproteinase (MMP) inhibitors, tissue inhibitors of metalloproteinases (TIMPs) (47). Like MMPs, meprins are generally synthesized in their inactive proforms, but they differ from MMPs in that they are not activated by organomercurials (47). Both meprin α- and β-subunits contain considerable N-linked oligosaccharide chains, which have been shown to be necessary for proper folding, oligomerization, stability, and proteolytic activity (42, 46, 47). In addition to N-linked carbohydrates, β-subunits also contain O-linked sugars that may regulate the proteolytic cleavage and secretion of the enzyme (56). The identity of the structures of the carbohydrate chains attached to the enzyme is not completely known. Trypsin, a serine protease, can activate meprins in vitro as well as in the intestinal tract; however, it is not known which protease(s) activates meprins in tissues lacking trypsin, such as the kidney. Trypsin cleaves the C-terminal to Arg or Lys, and the Arg-Asp cleavage in the meprin prosencephalium is important for activation (45). In the absence of trypsin expression in the kidney, trypsin-like proteases may activate meprin A. There are many potential protease candidates, including calpains, caspases, metalloproteinases, aminopeptidases, and kallikrein-related peptidases (KLKs) in the renal proximal tubule. Most of the KLKs are trypsin-like serine proteases and may activate meprin A in vivo and in vitro. KLK peptidases, including KLK1 and KLK4–KLK7, are expressed in the renal proximal tubules (23, 50, 67, 71). In fact, a recent study has reported that KLK4 and to a lesser extent KLK5 and KLK8 can activate human promeprin-β (5). Therefore, it is likely that during AKI pro-meprin A in the kidney is activated by one of the renal proximal tubular resident KLKs. Many other members of the astacin family are activated by furin (55). Human pro-meprin-α but not pro-meprin-β can be activated by plasmin (4, 68).
Substrates of Meprins and Implications in Biological Functions

To understand the role of meprins in normal physiological and pathological conditions, numerous target substrates of meprins have been identified (Table 1). Although meprins are capable of cleaving a wide variety of peptides and proteins in vitro, there is little information on the identity of meprin substrates under physiological or pathophysiological conditions. Bioactive peptides, including insulin, bradykinin, angiotensin II (14), luliberin, substance P, and α-melanocyte-stimulating hormone (α-MSH) (48, 73), are among a few substrates for meprin initially identified in in vitro studies. However, the biological relevance of these substrates in vivo under physiological or pathophysiological conditions is yet to be examined. Numerous other substrates of meprin A and meprin B are described in Table 1 as they were discovered.

The ability of meprin A to degrade ECM proteins was first described in 1994 when it was demonstrated that meprin A purified from rat kidney cortices was capable of cleaving basement components including collagen IV, laminin, nidogen, fibronectin, and gelatin (47, 84). Meprin A was found to be the major matrix-degrading enzyme in the proximal tubules (47, 84). Despite the importance of ECM components in cell attachment, proliferation, migration, and differentiation (54, 89), very little is known regarding alterations in the ECM of the renal tubular basement membrane in AKI. There was a marked reduction in renal tubular laminin following ischemia-reperfusion (IR) injury (83). When the EHS laminin/nidogen complex was incubated with meprin A purified from kidney cortex, a major 55-kDa fragment of nidogen was produced (84), suggesting that nidogen-1 in the basement membrane is relatively more susceptible to meprin A. A meprin-induced 55-kDa nidogen breakdown product was also detected in the urine of rats subjected to renal IR injury (84). A urinary nidogen fragment was detected much before the rise in serum creatinine, suggesting that this fragment may serve as an important early biomarker for IR injury (Herzog C, Marisiddiah R, Haun RS, Kaushal GP, unpublished observations). In addition, a meprin inhibitor and meprin-β knockout (KO) mice prevented the urinary excretion of the nidogen fragment (Herzog C, Marisiddiah R, Haun RS, Kaushal GP, unpublished observations), suggesting a pathophysiological link to the...
Table 1. Substrates of meprins

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Meprin Source and Isoform</th>
<th>Reference (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, bradykinin, angiotensin II</td>
<td>Mouse kidney meprin A</td>
<td>14</td>
</tr>
<tr>
<td>Leuiberin or luteinizing-hormone-releasing hormone (LHRH) and bradykinin, and substance P</td>
<td>Rat kidney meprin A</td>
<td>48, 73</td>
</tr>
<tr>
<td>Insulin B chain, oxytocin, substance P, bradykinin angiotensin I, and angiotensin II</td>
<td>Human small intestinal meprin</td>
<td>75</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Rat kidney meprin A</td>
<td>19</td>
</tr>
<tr>
<td>Parathyroid hormone (PTH)</td>
<td>Rat kidney meprin A</td>
<td>87</td>
</tr>
<tr>
<td>Collagen IV, laminin, fibronectin, gelatin, and nidogen</td>
<td>Rat kidney meprin A</td>
<td>48, 84</td>
</tr>
<tr>
<td>Catalytic subunit of protein kinase A</td>
<td>Rat kidney meprin A</td>
<td>18</td>
</tr>
<tr>
<td>Laminin 1 and laminin 5</td>
<td>Recombinant homomeric human meprin A</td>
<td>49</td>
</tr>
<tr>
<td>Bombesin, neurotensin, LHRH, bradykinin, α-melanocyte-stimulating hormone (α-MSH), substance P, PTH fragment 13–34, valosin, vasoactive intestinal peptide, and angiotensin I</td>
<td>Mouse meprin A (αβ)</td>
<td>8, 86</td>
</tr>
<tr>
<td>Gastrin 17, peptide YY, orocinulin, and kinetin</td>
<td>Recombinant human homomeric meprin A and mouse meprin B</td>
<td>8</td>
</tr>
<tr>
<td>Cereulin, secretin, glucagon, Gastrin-releasing peptide (GRP)-(14–27), neuropeptide Y, and SCCK8NH2</td>
<td>Both mouse meprin A (αβ) and meprin B</td>
<td>8</td>
</tr>
<tr>
<td>Collagen IV, nidogen-1, and fibronectin</td>
<td>Human recombinant homomeric meprin A and human meprin B</td>
<td>52</td>
</tr>
<tr>
<td>Pro-IL-1β</td>
<td>Rat meprin A (αβ), recombinant homomeric rat meprin A, recombinant human meprin B</td>
<td>33, 34</td>
</tr>
<tr>
<td>GRP, glucagon, ghrelin, PTH, secretin, LHRH, CCK8 nonsulfated, substance P, bradykinin, neurotensin, and α-MSH</td>
<td>Recombinant human homomeric meprin A and mouse meprin A</td>
<td>16</td>
</tr>
<tr>
<td>Pro-IL-18</td>
<td>Recombinant human meprin B</td>
<td>2</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Recombinant human meprin B</td>
<td>39</td>
</tr>
<tr>
<td>Processing of procollagen III</td>
<td>Recombinant human meprin B and homomeric meprin A</td>
<td>51</td>
</tr>
<tr>
<td>Pro-kallikrein-7 (Pro-KLK7)</td>
<td>Recombinant human meprin B</td>
<td>64</td>
</tr>
<tr>
<td>Villin and actin</td>
<td>Recombinant rat meprin B and mouse homomeric meprin A</td>
<td>66</td>
</tr>
<tr>
<td>α- and γ-Epithelial Na+ channel (ENaC) subunits</td>
<td>Recombinant rat meprin B</td>
<td>26</td>
</tr>
<tr>
<td>Amyloid precursor protein</td>
<td>Recombinant human meprin B</td>
<td>44</td>
</tr>
<tr>
<td>Pro-ADAM-10 (a disintegrin metalloproteinase)</td>
<td>Recombinant human homomeric meprin A and meprin B</td>
<td>43</td>
</tr>
<tr>
<td>Pro-MMP-9</td>
<td>Recombinant human homomeric meprin A and meprin B</td>
<td>27</td>
</tr>
</tbody>
</table>

TGF, transforming growth factor.

The ability of meprins to degrade ECM components has shown that meprin isoforms can activate MMP-9 in vitro studies (27); however, more studies are needed to determine whether activation of MMP-9 by meprins provides a biological link with classic metalloproteinases in vivo that regulate the ECM components. As described in Table 1, meprins are also capable of proteolytically processing bioactive peptides, cytokines, and peptide hormones. Recent studies have demonstrated that all meprin isoforms are able to produce biologically functional IL-1β (33, 34) and IL-18 (2) from their inactive proforms, suggesting that meprins may play an important role in inflammatory processes in addition to their ability to degrade ECM components.

E-cadherin, a key transmembrane glycoprotein in adherins junctions in epithelial cells, was recently shown to be cleaved by meprin β or heterodimeric meprin A but not by meprin α. When stably transfected MDCK or Caco-2 cells expressing meprin β or meprin α and β together were treated with trypsin to activate meprin zymogen in situ, 120-kDa E-cadherin was cleaved to produce a 97-kDa fragment (39). The formation of the 97-kDa fragment was inhibited in the presence of actinin, suggesting that the cleavage is specific for meprin isoforms. It was further shown that the adhesive strength of meprin-expressing cells was markedly reduced compared with nonexpressing cells. However, this cleavage has yet to be established in vivo during AKI. The integrity of adherins junction proteins is important in the maintenance of functional tight junctions, cell-cell interaction, cell polarization, and migration; thus their cleavage can promote cell detachment, impair epithelial barrier function, and cause damage to polarized epithelium during renal injury. Although MDCK cells...
provided an interesting model for studying cadherins as targets of meprins and their role in cell-cell adhesion, further studies will be required to determine whether cadherins and other proteins in adherens junctions are targets of meprins in vivo during AKI and whether meprins promote cell detachment through cleavage of focal adhesion proteins. It was recently demonstrated that actin and its associated actin-bundling protein villin are capable of binding to the C terminus of meprin-β, and both of these proteins are degraded by meprin A as well as meprin B (66). The degraded fragments were not detected in meprin KO subjected to IR injury. These studies provide evidence that meprin A-mediated cleavage of actin and villin may contribute in part to the disruption of the dynamic cytoskeletal structures in the microvilli core of the brush borders that leads to apoptosis and necrosis during AKI. Meprin β promoted cleavage of α- and γ-subunits of the epithelial sodium channel (ENaC) at sites close to the second transmembrane domain in the extracellular domain of each channel subunit. Although several substrates of meprins have been identified, only IL-1β, IL-18, ECM component nidogen, ENaC tumor growth factor α (TGF-α), and amyloid precursor protein (APP) (Table 1) and others identified by a proteomic approach, terminal amine isotopic labeling of substrates (TAILS), including extracellular matrix proteins and growth factors, have been demonstrated as target substrates in vivo studies (43). However, further studies are required to identify meprin substrates in vivo in AKI and other pathophysiological conditions.

**Meprin A Redistribution and Role in AKI**

Many studies have reported a role of meprin A in the pathogenesis of AKI induced by IR and cisplatin nephrotoxicity. After renal IR, a marked alteration in meprin A distribution from its usual linear staining of the brush-border membranes to reach the underlying basement membrane has been reported (15, 17, 84). The redistribution of meprin A after renal IR is illustrated in Fig. 2B. Similarly, altered distribution of meprin A has been observed in cisplatin (35) and glycerol (80) experimental models of AKI. Studies using double-immunofluorescence staining of kidney sections with meprin A (red) and Na-K-ATPase (green) or nidogen basement membrane confirmed that meprin A is exclusively localized to brush-border membranes of the proximal tubules and is redistributed in the injured kidney during IR and cisplatin nephrotoxicity. Although some reports on the immunostaining results have suggested the possibility of intracellular localization of meprin A during IR injury, more studies are required to prove meprin localization in the cytoplasmic compartments during renal injury. Meprin A, that is normally restricted to the brush-border membranes of tubules in the S3 segment, may be detrimental in renal injury due to its altered localization and enormous destructive potential. Meprin A redistribution during IR has been attributed to result in cellular damage and promote an inflammatory response (15, 17). In addition to altered localization toward the basolateral side, meprin A is also secreted in the urine during AKI (35, 38), suggesting that meprin A is shed under pathological conditions. ADAM-10 has been recently identified as a putative sheddase responsible for the release of membrane-associated meprin A (Herzog C, Haun RS, Ludwig A, Shah SV, Kaushal GP, unpublished observations). The secretion of meprin in the human tubular lumen has been studied in urinary tract infections (UTI). High meprin levels in human urine have been correlated with an active UTI (12).

The role of membrane-associated-meprin A in AKI was first investigated using inbred strains of mice expressing high levels of meprin A (strains C57BL/6 and DBA/2) and those expressing relatively low levels of meprin A (strains C3H/He and CBA). In experimental models of IR- and glycerol-induced nephrotoxicity, C3H/He and CBA mice displayed a marked reduction in tubular necrosis compared with those that expressed high levels of meprin A (80). In a model of cisplatin nephrotoxicity, low meprin A-expressing mice (C3H/He) exhibited significantly less tubular necrosis, serum creatinine, and blood urea nitrogen (BUN) values compared with high meprin A-expressing mice (C57BL/6 mice) (35). Also, actinonin, a potent and a pharmacological inhibitor of meprin A, significantly decreased AKI due to IR and cisplatin nephrotoxicity as reflected by improvement in histological damage and reductions in BUN and serum creatinine levels (17, 35). Hypoxia-reoxygenation injury as measured by LDH release in renal slices was also prevented by the meprin inhibitor actinonin (17). Moreover, active meprin purified from rat kidney was found to be cytotoxic in renal tubular epithelial cells in culture, further suggesting a detrimental role of meprins during renal injury (17). Consistent with these findings, a recent study showed that meprin β KO mice are protected from renal IR injury (15). Meprin β KO mice displayed significantly less infiltration of inflammatory cells, improved renal function, and histology compared with wild-type (WT) mice. Urinary levels of inflammatory cytokines IL-6 and KC (CXCL1) were significantly higher in WT compared with meprin β KO mice. In one study, it was shown that preischemic treatment with actinonin (10 or 30 mg/kg iv) dose dependently attenuated IR-induced renal injury in male rats, but failed to improve renal injury in female rats (78). The findings that meprin A contributes to the pathology of renal injury are also supported by a recent study that meprin A is capable of cleaving the cytoskeleton components actin and villin following IR injury (66). Actin cytoskeleton disruption is known to be associated with IR injury (63). Since meprins are highly glycosylated proteins, the oligosaccharide chains of meprin A may serve as important ligands for mannan binding proteins (MBP) present in proximal tubules (37). A recent study suggested that the interaction between serum-specific MBP and meprins led to complementary C3b accumulation that may contribute to IR injury (36).

The role of meprins has also been examined recently in experimental models of sepsis-induced AKI. Since sepsis symptoms in patients are identified only when sepsis is already established, the effect of the meprin A inhibitor actinonin was examined before and after the onset of sepsis. In an experimental model of cecal ligation and puncture (CLP), the meprin A inhibitor improved peritubular renal capillary perfusion not only when administered before the onset of sepsis injury but also when administered after the onset of injury. Both early and late treatment with the meprin A inhibitor markedly reduced sepsis-induced IL-1β production and ameliorated sepsis-induced loss in renal function and histology (85). The role of meprin A inhibition has also been examined in the outcome of LPS-induced AKI using meprin α KO and meprin β KO mice. During the course of LPS injury, while meprin α KO mice displayed a marked decrease in the levels of IL-1β and TNF-α,
meprin β KO mice did not exhibit significant changes in these cytokines compared with WT mice (88). The endotoxicemic injury in response to LPS resulted in a significant decline in renal function and decreased nitric oxide levels in meprin-β KO but not in meprin-α KO mice. In addition, meprin-β KO mice displayed marked bladder inflammation with leukocyte infiltration and edema in the bladder. These studies suggested that homomeric meprin A but not meprin B is involved in renal injury due to LPS. It would be of interest to determine whether membrane-associated meprin A composed of α- and β-subunits is also involved in renoprotection from CLP-induced AKI.

This review highlights significant progress in discerning the structure, distribution, and substrates of meprin A, and particularly, the role of meprin A in experimental models of AKI. Recent progress in the identification of in vitro substrates will provide more insight into the identification of important targets of meprin A in vivo during the development of AKI and other pathophysiological conditions. In addition, this review emphasizes the need to characterize the role of meprin A in leukocyte infiltration, loss of renal epithelial cells, and the mechanism of meprin shedding during AKI. The identification of a specific ADAM metalloproteinase involved in the shedding of meprin A in vivo will also identify a novel therapeutic target in addition to meprin A in AKI.

ACKNOWLEDGMENTS

The authors thank Cindy Reid and Sarah Kaushal for technical editing support.

GRANTS

This work was supported by grants from the Veterans Administration (VA Merit) to G. P. Kaushal, R. S. Haun, and S. V. Shah and the National Institutes of Health (DK-081690) to G. P. Kaushal.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: G.P.K. provided conception and design of research; G.P.K., R.S.H., and C.H. analyzed data; G.P.K. and S.V.S. interpreted results and revised manuscript; G.P.K. and S.V.S. approved final version of manuscript.

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