The ins and outs of angiotensin processing within the kidney

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The kidney is a key target for the diverse components of the renin-angiotensin-aldosterone system that include prorenin/renin, angiotensin II (ANG II), ANG-(2–8) (ANG III), ANG-(1–7), ANG-(3–8), ANG-(1–9), and aldosterone (2, 3, 6, 10). The kidney also comprises an intrinsic renin-angiotensin system (RAS) particularly within the proximal tubule epithelium capable of producing bioactive peptides to activate their respective receptors (R) in a paracrine or autocrine manner (2, 6, 10). Currently, the renal RAS can be functionally partitioned into at least two arms based on the distinct processing enzymes and receptors that comprise the ANG II-AT1 receptor and ANG-(1–7)-AT7/MasR axis (2, 3, 7, 10, 14). In general, these two pathways exhibit opposing effects in the kidney and may antagonize the actions of one another (2, 3). Within the tubular system of the kidney, ANG II stimulates the AT1 receptor to attenuate oxidative stress, and stimulates cellular phosphatases to inhibit mitogen-activated kinase pathways such as chymase to form ANG-(1–5) (2). ACE inhibitors increase circulating levels of ANG-(1–7) by preventing the rapid metabolism of the peptide, as well as shifting the processing of ANG I to ANG-(1–7) by the endopeptidase nephrilysin (NEP) (2, 13, 14). All three enzymes are classified as metallopeptidases

ACE2 efficiently metabolizes ANG II to ANG-(1–7) and may markedly alter the functional signature of the RAS (2). ACE2 is a monoenzyme that does not continue to metabolize ANG-(1–7) due to the COOH-terminal proline; however, ACE hydrolyzes the Ile5-His6 bond of ANG-(1–7) to form ANG-(1–5) (2). ACE inhibitors increase circulating levels of ANG-(1–7) by preventing the rapid metabolism of the peptide, as well as shifting the processing of ANG I to ANG-(1–7) by the endopeptidase nephrilysin (NEP) (2, 13, 14).

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Fig. 1. Proposed scheme for the extracellular and intracellular processing of angiotensin peptides within renal proximal tubules. Extracellular metallopeptidases, angiotensin-converting enzyme (ACE), ACE2, and nephrilysin (NEP) process ANG I to ANG II or ANG-(1–7) (ANG 7) that subsequently bind to receptors on the cell surface. Intracellular peptides include thimet oligopeptidase (TOP) to process ANG I to ANG-(1–7) or ACE-independent (non-ACE) pathways such as chymase to form ANG II. An ANG-(1–7) endopeptidase (A7-EP) hydrolyzes the peptide to ANG-(1–4) (ANG 4); the peptide may be secreted for extracellular metabolism of ANG-(1–7) in addition to ACE-dependent metabolism of ANG-(1–7) to ANG-(1–5) (ANG 5). The precursor angiotensinogen (Aogen) may be internalized by the tubules from extracellular sources or arise from intracellular synthesis.
with membrane-anchoring domains that orient their active sites on the extracellular cell surface to process substrates within the glomerular filtrate, interstitial fluid, cerebrospinal fluid (CSF), or the blood (Fig. 1). These peptidases comprise the extracellular pathway for the formation of ANG II and ANG-(1–7) to subsequently bind to AT$_1$R or AT$_2$R on the cell surface and activate various signaling pathways (Fig. 1). ACE, ACE2, and NEP also contribute to the metabolism of both peptides to either inactive forms or, in the case of ANG-(1–7), a metabolite that functionally opposes the ANG II-AT$_1$R axis (2, 13).

In addition to the extracellular processing of peptides, there is compelling evidence for the intracellular expression of both ANG II and ANG-(1–7) in the kidney and other tissues (1–8). The tissue expression of angiotensins may indeed lead to their subsequent release into the extracellular space; however, evidence of intracellular AT$_1$R, AT$_2$R, and AT-R may portend for processing pathways that provide an intracellular source of ANG II or ANG-(1–7) as potential receptor ligands (1–8). In glomerular or diabetic conditions, ANG II content is increased in various cell types and appears to reflect the contribution of non-ACE-dependent pathways such as chymase or cathepsins (5, 8, 11). Elucidation of the intracellular pathways for ANG-(1–7) formation and metabolism within the intracellular compartments of the kidney and other tissues is less clear. Costa-Neto and colleagues (12) reported that the soluble enzyme thimet oligopeptidase (TOP) processed ANG I predominantly to ANG-(1–7) in the rat hippocampus independent of ANG II. TOP was also identified as the primary activity that directly converted ANG I to ANG-(1–7) without the prerequisite formation of ANG II in nuclei isolated from the NRK-52E renal epithelial cells (1). In regard to ANG-(1–7) metabolism, our recent studies reveal a soluble endopeptidase that degrades ANG-(1–7) to the inactive metabolite ANG-(1–4) in the CSF and brain medulla of sheep (3, 9). The purified peptidase exhibits a preferred specificity for ANG-(1–7) compared with other angiotensins; the rate of ANG-(1–7) processing to ANG-(1–4) was 10- to 20-fold higher than for ANG II and ANG I, whereas other peptides including bradykinin, neurotensin, and apelin were not hydrolyzed by the enzyme following a 24-h incubation (9). The ANG-(1–7) endopeptidase also exhibits subnanomolar affinity (IC$_{50}$ = 0.8 nM) against the inhibitor JMV-390 that is markedly lower than that reported for other metallopeptidases such as NEP, neurolysin, TOP, and ACE (9). Preliminary studies now identify the ANG-(1–7) endopeptidase in the soluble fraction of isolated proximal tubules of the sheep kidney, as well as in the human proximal tubule HK-2 cell line (15). Both peptidase activities in the tubules and HK-2 cells exhibit a similar high affinity for the JMV inhibitor to block the conversion of ANG-(1–7) to ANG-(1–4) (15). Moreover, endopeptidase activity was evident in the serum-free media of the HK-2 cells, suggesting that the enzyme is released to participate in the extracellular metabolism of ANG-(1–7) within the filtrate or the interstitial compartment of the kidney (15).

Perspectives and Significance

Functional partitioning of the RAS is facilitated in part through multiple peptidase pathways that occur downstream from the initial processing of angiotensinogen by renin and likely reflects their discrete cellular localization and relative affinities for peptides. Fetal glucocorticoid exposure is one example of in utero programming events that remarkably influence the RAS in adults through altered expression of distinct peptidase components in the kidney, circulation, and brain (3). Although additional characterization of the ANG-(1–7) endopeptidase regarding the enzyme’s specificity and regulation is warranted, therapeutic approaches that target both intracellular and extracellular pathways to enhance the “ANG-(1–7) to ANG II tone” that include reduced metabolism of ANG-(1–7) may provide additional renoprotection in diabetes, hypertension, and fetal programming events.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: B.A.W., A.C.M., and M.C.C. conception and design of research; B.A.W. performed experiments; B.A.W., A.C.M., E.M.A., and M.C.C. analyzed data; B.A.W., E.M.A., and M.C.C. interpreted results of experiments; B.A.W., A.C.M., E.M.A., and M.C.C. drafted manuscript; B.A.W., A.C.M., and M.C.C. edited and revised manuscript; B.A.W., A.C.M., E.M.A., and M.C.C. approved final version of manuscript; A.C.M. and M.C.C. prepared figures.

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