**Prolyl carboxypeptidase: a forgotten kidney angiotensinase. Focus on “Identification of prolyl carboxypeptidase as an alternative enzyme for processing of renal angiotensin II using mass spectrometry”**

Juan Carlos Q. Velez  
Division of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina  
Submitted 25 March 2013; accepted in final form 26 March 2013

HYPERTENSION, which affects one-third of the population, is commonly treated with inhibitors of ANG II production or action, as this hormone triggers vasoconstriction and raises extracellular volume by increasing sodium reabsorption along the nephron. ANG II, an octapeptide [ANG-(1–8)], emanates from the enzymatic cleavage of the ~50-kDa precursor angiotensinogen by renin and angiotensin-converting enzyme (ACE). The ongoing study of the renin-angiotensin system continues to unveil novel participants, as well as new roles for established participants, adding further complexity to the understanding of this important hormonal system (7). Among those relatively newly discovered elements, ACE2 (EC 3.4.17.23) has been extensively studied because of its involvement in the conversion of ANG II to ANG-(1–7) by eliminating the last amino acid of the peptide at the carboxy terminus. Because ANG-(1–7) may act counter to the actions of ANG II, that is, as a vasodilator, an antiproliferative, and a natriuretic hormone (1), several laboratories have focused on exploring whether ACE2 has protective effects in models of cardiac and renal disease. In this issue, Grobe et al. (3) present evidence that, in addition to ACE2, prolyl carboxypeptidase (PCP, EC 3.4.16.2) should be considered an important enzyme mediating the conversion of ANG II to ANG-(1–7) in the kidney. Using state-of-the-art mass spectrometric techniques and genetically engineered mouse models, the authors carefully showed that PCP is an active ANG II-metabolizing enzyme in the cortex of the rodent kidney. This report expands on their recent work (2) and is an active ANG II-metabolizing enzyme in the cortex of the engineered mouse models, the authors carefully showed that PCP participating in the characterization of ANG (1–7) as a “renoprotective” peptide (11). Alternatively, the importance of the conversion of ANG II to ANG-(1–7) by ACE2 and/or PCP may relate to the resulting initiation of a cascade intended to degrade ANG II. On that note, it should be acknowledged that other routes of ANG II metabolism exist in the kidney. Specifically, at the amino terminus, ANG II is converted to ANG III [ANG-(2–8)] by APA, whereas ANG III is subsequently converted to ANG IV [ANG-(3–8)] by aminopeptidase N (EC 3.4.11.2). In addition, upon cleavage of the octapeptide at an internal bond, ANG II is converted to ANG-(1–4) by neutral endopeptidase (EC 3.4.24.11; Fig. 1).

The concept of pH dependency as a determinant of enzymatic activity responsible for ANG II metabolism is provocative. Grobe et al. (3) postulate that PCP could be a particularly important enzyme at acidic pH, whereas ACE2 might be more relevant in a neutral or alkaline environment. It was previously reported that the optimum pH for PCP is close to 5 (10). While ACE2 is reported to maintain activity in acidic conditions, this enzyme is also pH sensitive (50-kDa precursor angiotensinogen by

---

**Address for reprint requests and other correspondence:** J. C. Velez, Division of Nephrology, Medical Univ. of South Carolina, 96 Jonathan Lucas St., CSB Rm. 826F, Charleston, SC 29425 (e-mail: velezj@musc.edu).

http://www.ajpcell.org 0363-6143/13 Copyright © 2013 the American Physiological Society
study shows that kidney homogenates of ACE2-deficient mice show reduced conversion of ANG II to ANG-(1–7) only at basic pH (3). Thus, if the present studies reflect true physiology, one could postulate that PCP may be an active ANG II-processing enzyme at the level of the collecting duct where the intraluminal urinary pH is known to be acidic. However, the use of crude kidney lysates to investigate the effect of pH on ACE2 and PCP activity poses the question whether the observation of pH dependency relates to catalysis of ANG II within the tubular lumen, in the proximity of the cell membranes, or corresponds to intracellular lysosomal proteases. Furthermore, even though the kidney is known to host a substantially higher concentration of ANG II than plasma, the substrate concentrations used by Grobe et al. were largely above the range reported in the literature. Consequently, it remains to be determined whether their findings apply to the conditions of endogenous in vivo ANG II metabolism.

This study revives the dilemma about the intrarenal localization(s) of ANG II metabolism. The sophisticated technique of matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometric-based tissue imaging offers promise as a method to spatially resolve endogenous peptides in various organs. However, the highest resolution provided by MALDI-TOF [~10–50 μM (6)] is insufficient to accurately map peptide abundances to specific cells or regions. In the case of the kidney cortex, tissue MALDI-TOF images do not differentiate whether the peptide of interest localizes in the glomerular or the tubular compartment. This limitation is likely to be circumvented by future technological advancements. At the other end of the technical spectrum, we have learned a great deal about intrarenal enzymatic and transporter profiles from manual and enzymatic dissection and isolation of tubule fragments and glomeruli. Recently, tubule isolation and assay have been facilitated by genetic fluorescent labeling of specific tubules (4). Tubule fractionation coupled to analysis of ANG II-metabolizing enzymes or direct analysis of ANG II metabolites could be exploited to provide insight into ANG II metabolism along the nephron as well. By understanding where these peptides are produced, we can move ahead to understand their physiological activities and relevance to fluid and electrolyte, as well as blood pressure, homeostasis.

In summary, the report by Grobe et al. (3) deservedly brings PCP back “on the radar” in the study of intrarenal ANG peptide metabolism. Future studies are necessary to confirm the role of PCP in endogenous ANG II metabolism in the kidney. Similarly, more insights into the influence of intrarenal ANG II on the regulation of PCP in normal and disease states would be of interest.

GRANTS

J. C. Velez is supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-080944-05 and Dialysis Clinics Incorporated.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

J.C.V. prepared the figures; J.C.V. drafted the manuscript; J.C.V. edited and revised the manuscript; J.C.V. approved the final version of the manuscript.

REFERENCES