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CLINICAL IMPLICATIONS

The renin-angiotensin system and prolylcarboxypeptidase

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The principal active peptide of the renin-angiotensin system is angiotensin II (Ang II), a vasoconstrictor peptide and promoter of atherosclerosis. However, perhaps to counter these effects, the renin-angiotensin system also consists of peptides and receptors that increase nitric oxide release from the endothelium and decrease nicotinamide adenine dinucleotide phosphate oxidase-related superoxide production [1]. Two peptides, Ang (1–7) and alamandine, are vasodilators. They activate the nitric oxide pathway via different receptors in the endothelium. The sequence of alamandine is very similar to Ang (1–7), differing only by the presence of an alanine residue in place of an aspartate residue in the amino terminus. This rather unexpected "alternative" arm of the renin-angiotensin system [2] also involves the monocarboxypeptidase angiotensin-converting enzyme-2 (ACE2) that counterbalances the effects of the classical renin-angiotensin system through the degradation of Ang II and also generates Ang (1–7). ACE2 is highly expressed in tissues of cardiovascular relevance including the heart, blood vessels, and kidney.

The lysosomal pro-X carboxypeptidase (PRCP) cleaves C-terminal amino acids linked to proline in peptides such as Ang II, Ang III, and des-Arg9-bradykinin. The cleavage occurs at acidic pH; however, the enzyme activity is retained with some substrates at neutral pH. The enzyme is also an activator of cell matrix-associated prekallikrein [3]. Thus, there are several pathways to Ang (1–7) production, including ACE2 and PRCP. Ang II, Ang (1–7), kallikrein, and bradykinin all influence the arterial blood pressure. There are several routes connecting PRCP to hypertension. Association of polymorphisms in prolylcarboxypeptidase with essential hypertension has been described in a Chinese Han population [4]. Furthermore, investigators have identified PRCP as a regulator of blood vessel homeostasis. Invasive telemetric blood pressure monitoring showed that *PRCP* gene-targeted mice had significantly elevated blood pressure [5].

Another potentially important enzyme in this spectrum of interactions is the zinc-dependent metalloprotease neprilysin (NEP). NEP cleaves numerous vasoactive peptides, some with mainly vasodilating effects (natriuretic peptides, adrenomedullin, bradykinin) and others with mainly vasoconstrictor effects (angiotensin I, endothelin-1). NEP converts the decapeptide angiotensin I to Ang (1–7). Recently, the combination of NEP and angiotensin receptor inhibitors (sacubitril/valsartan), also known as ARNI (brand name Entresto), has shown good results in heart failure with reduced ejection fraction, and a multitude of ongoing studies are set to prove its value across the heart failure spectrum. Domenig et al. recently assayed murine kidney angiotensins in wildtype and ACE2 knockout mice by means of a renin-angiotensin system-fingerprint analysis [6]. Moreover, they also investigated the ex vivo metabolism of spiked Ang I or Ang II in the presence and absence of selective inhibitors in renal extracts by mass spectrometry. Matrix-assisted laser desorption/ionization (MALDI)-imaging was used to investigate renal location of angiotensin metabolism. Metabolic analysis revealed a major role of PRCP in Ang (1–7) formation in mice. They also identified neprilysin (NEP)-dependent conversion of Ang I to Ang (1–7) to be the main pathway of Ang (1–7) formation in murine kidneys, which was mainly located in the renal cortex, as confirmed by MALDI-imaging. The authors also tested the potential relevance of their findings for antihypertensive and renoprotective therapy in humans by analyzing Ang metabolism in human living donor renal biopsies. In

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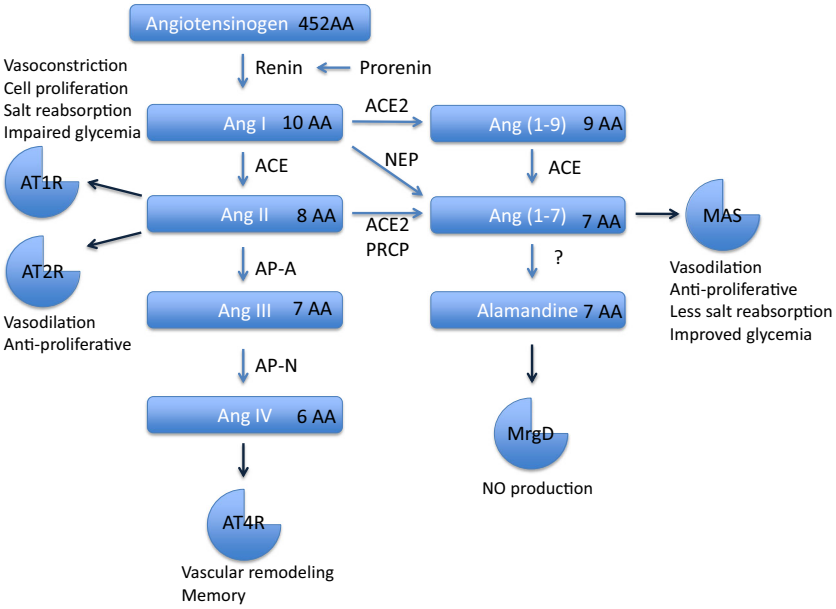

Fig. 1 The renin-angiotensin system. Not all of the putative functions are documented in detail, and the schema is subject to change.
AA amino acids,
ACE angiotensin-converting enzyme,
ATR angiontensin receptor,
NEP neprilysin,
AP aminopeptidase,
PRCP prolylcarboxypeptidase,
NO nitric oxide,
MAS G protein-coupled receptor,
MrgD mas-related G protein-coupled receptor D

contrast to mice, they found that the Ang II-degrading activity of ACE2 directed the renin-angiotensin system to the alternative Ang (1–7) axis, as compared to that of PRCP. Figure 1 shows a scheme of these various pathways.

In this issue of J Mol Med, Maier and colleagues report on the impact of PRCP deficiency on Ang II metabolism, blood pressure, cardiac, and renal histology in two different strains (C57BL/6 and FVB/N) of PRCP gene-trap mice [7]. Gene trapping is a high-throughput approach that is used to introduce insertional mutations. Gene traps simultaneously inactivate and report the expression of the trapped gene at the insertion site. Both strains had a modest increase in blood pressure and left ventricular hypertrophy (LVH). An increase in glomerular size and mesangial expansion was also reported by the authors in the C57BL/6 strain of mice. Despite absence of PRCP, no difference in plasma or cardiac levels of Ang (1–7) could be identified between PRCP gene-trap and control mice with either strain. Chronic Ang II infusion increased blood pressure and worsened LVH; however, under those conditions, no constitutive differences between knockout and control mice were observed. The authors concluded that PRCP deficiency is associated with elevated blood pressure and cardiac alterations including LVH and renal defects independent of systemic or cardiac Ang II and Ang (1–7). The authors then performed ex vivo experiments on PRCP-mediated Ang II to Ang (1–7) conversion. The conversion did not occur at all at pH 7.4, but only at an acidic pH. PRCP was localized in α-intercalated cells of the kidney collecting tubule. Since this site operates at low pH, the authors speculate that Ang II conversion in the renal collecting duct could influence sodium reabsorption and thereby affect blood pressure. Unfortunately, the authors did not directly test this hypothesis.

Salt-sensitive hypertension in humans and animal models is associated with increased cardiovascular and renal morbidity. In animal models, numerous mechanisms have been elucidated, but in humans, the mechanisms are yet poorly understood [8, 9]. A direct intrarenal action of the renin-angiotensin system would be an important demonstration. Carey recently reviewed the relevance of the intrarenal renin-angiotensin system in detail [10]. The renin-angiotensin system within the kidney functions independently of the more appreciated systemic renin-angiotensin system and is particularly activated in hypertension. The system can auto-amplify itself to multiply its actions. The Ang III/AT2 receptor pathway, the ACE-2/Ang (1–7)/Mas receptor pathway, and possibly the newly described alamandine/MrgD pathway offer further avenues of exploration. The current contribution implying that urinary acidification could influence degradation of Ang II to Ang (1–7) should be vigorously pursued.

Respectfully,
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