In this issue of *JASN*, Huang *et al.* (1) report an immunohistochemical study in patients with diabetic nephropathy. While only faint expression of chymase was seen in mesangial cells and vascular smooth muscle cells (VSMC) of normal human kidneys, marked expression of chymase by mesangial cells and VSMC was seen in kidneys of diabetic patients with nephropathy, particularly when hypertension was present. Strong chymase immunoreactivity was found in fibrotic tissue of the mesangium, in atherosclerotic vascular lesions, and in areas of interstitial fibrosis.

The findings are potentially of great relevance, although the authors do not provide evidence of functional activity of chymase. The authors correctly point out that upregulation of angiotensin-converting enzyme (ACE)–dependent pathways of generation of AngII may have important implications for treatment strategies.

As a background, it may be useful to provide some information concerning chymase in general.

What is chymase? Human chymase is a protease that is synthesized as an inactive zymogen and has now been characterized at atomic resolution (2). Exciting phylogenetic information suggests that the reconstructed ancestral molecule of chymase had already substantial angiotensin II–forming activity pointing to a long evolutionary history (3,4). The chymase locus comprises a number of serine protease enzymes that all have no enzymatic activity in the normal state but are activated immediately upon release into the extracellular matrix. The leader peptide is clipped off, and they are then secreted. Chymase is a distinct enzyme within the larger family of mast cell serine proteases. They display highly selective hydrolysis of specific peptides, in the instance of chymase PHE-8, HIS-9 in angiotensin I to form angiotensin II.

What are the known actions of chymase? Chymase is highly expressed in mast cells, and an association between genetic variants of mast cell chymase and skin disease, e.g., eczema, has been noted (5). Chymase plays a role in angiogenesis by upregulating VEGF (6). Angiogenesis (7), including angiogenesis concerning chymase in general.

What is the role of chymase in humans? For a long time non–ACE-dependent pathways of generation of AngII have been suspected. As a case in point, a recent study on human internal mammary arteries evaluated the conversion of AngI to AngII in the presence of captopril with or without a chymase inhibitor (8). Chymase is highly expressed when vessels are damaged, e.g., after balloon injury. This type of vascular proliferation is inhibited both by angiotensin II receptor antagonists and by specific chymase inhibitors, suggesting that the effect is mediated via ACE-independent generation of angiotensin II (9,10).

What techniques do we have to study chymase? Apart from measuring chymase activity or ACE-independent generation of AngII, a number of interesting tools have recently become available. Wei *et al.* (11) studied mice with homozygous disruption of the ACE gene (–/–), heterozygous mice (+/–), and wild-type mice. AngII concentrations and AngII/AngI ratios in the kidney did not differ among genotypes, while plasma AngII concentration was extremely low. This observation suggests that chymase provides an important mechanism to maintain steady state AngII concentration in tissues but not in plasma. Conversely, transgenic mice produced by microinjection of chymase mRNA into the heart and other tissues with selective overexpression by a promoter-fusion gene had excessive AngII concentrations in the heart causing significantly increased metalloproteinase (MMP-9) activities and decreased collagen I synthesis (12). This observation illustrates the potential role of chymase in heart remodeling. Finally, while chymostatine has long been known to selectively inhibit chymase activity, a whole new generation of orally active low-molecular weight chymase inhibitors has recently become available, e.g., TEI-E-548, which was shown to increase survival of hamsters with myocardial infarction despite having no effect on the infarction size (13); BCEAB, which improved indices of LV function in cardiomyopathic hamsters (14), possibly by interfering with the activation of transforming growth factor β; and finally NK-3201, which was shown to suppress intimal hyperplasia after balloon injury (15) or to prevent peritoneal adhesions after intraabdominal trauma (16). The availability of such powerful tools will help to resolve some of the remaining uncertainties about the in vivo role of chymase.

What is the role of chymase in human animals? For a long time non–ACE-dependent pathways of generation of AngII have been suspected. As a case in point, a recent study on human internal mammary arteries evaluated the conversion of AngI to AngII in the presence of captopril with or without a chymase inhibitor (17). The long and the short of it was that AngII-mediated effects were more effectively inhibited by AngII antagonism than by ACE inhibition, pointing to the presence of alternative AngII-forming enzymes. The field is rendered difficult by substantial species and organ differences. For instance, Jin *et al.* (18) examined the ratio of ACE-dependent to chymase-dependent AngII formation in various species. In dog renal arteries, about 65% of the AngII-induced contraction was suppressed by chymase inhibition, while it was completely

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suppressed by an ACE inhibitor in rat arteries. The relative importance of chymase may further be altered by vascular and tissue pathology. As a case in point, Ohishi et al. (19) examined the expression of AngII in atherosclerotic plaques and their relation to ACE and chymase respectively using immunohistochemistry. Chymase was expressed in the cytosol of mast cells, but immune double-staining did not show colocalization of AngII and chymase (19), illustrating the difficulty of drawing conclusions about a functional role from immunostaining, a caveat that may also apply to the above study in diabetic kidney disease. In view of the species differences, it is important that an in vivo role of chymase in humans has been documented in a simple but informative system, i.e., venoconstriction of the vessel hand vein with a peptide analogue of AngI in the presence or absence of captopril. Using the ACE-resistant peptide, which is a specific substrate for chymase, the authors arrived at the conclusion that a non-ACE pathway capable of generating AngII exists in human veins in vivo (20).

What information is available on chymase in the kidney? In a series of studies Hollenberg et al. (21,22) investigated the maximal renal hemodynamic response, particularly the increase in ERPF, to ACE inhibitors, angiotensin receptor blockers, and renin inhibitors. ACE inhibition was least effective. Hollenberg et al. concluded that in the kidney virtually all AngII generation is renin-dependent, i.e., inhibited by renin inhibitors, but that at least 40% of AngI is converted to AngII by pathways other than ACE. At least the localization of chymase in VSCM of the renal vasculature, as found by Huang et al., would put the enzyme into the right place. Nevertheless, the faint expression in VSCM of normal kidneys would be difficult to reconcile with a major functional role of chymase in the control of renal plasma flow (21,22). Indeed Uurate et al. (23) had used Western blot and enzymatic assays and came to the conclusions that in human kidneys the activity of chymase was very low. Consequently, a potential role of other non-ACE enzymes is not definitely excluded. If the above-mentioned specific chymase inhibitors are shown to be safe, specific, and effective in humans, some of these problems may become clarified.

At least in the dog kidney, using the above-mentioned ACE-resistant analogue (20), which yields AngII by chymase, Murakami et al. (24) showed an increase of renal AngII concentration and selective, relatively modest, vasoconstriction of arterioles in the dog kidney. In the rat kidney, heterogeneity of afferent arterioles to inhibition of intracellular calcium changes by ACE and chymostatin, respectively, in response to AngI were recently documented by Marchetti et al. (25).

Expression of chymase in hamsters is thought to be strikingly analogous to humans. In this context, it is of interest that in the two-kidney/one-clip model of hypertension in hamsters, vascular chymase was not activated (26). Tokuyama et al. (27) investigated the same issue in unilateral renal artery stenosis in dogs and came to the conclusion that chymase activity is enhanced in the clipped kidneys, whereas ACE-mediated AngII generation is responsible for elevated AngII in the non-clipped kidneys.

In renal pathology, chymase expression has been studied in rejected grafts (28) and in IgA nephritis (29). In both situations, a close relation was found between interstitial fibrosis and mast cells, which are known to have high chymase concentrations. In future studies, it will be worthwhile to specifically look for mast cells in renal studies on chymase. With respect to potential mechanisms by which chymase is injurious to the kidney, it is of interest that human chymase cleaves big endothelin at the Tyr-31-Gly-32 bond, thus producing 31–amino acid endothelin. Generation of the known mesangial cell mitogen endothelin might be a potent mechanism to promote renal pathology via AngII independent pathways (30). Many issues are obviously still unresolved, but this field promises to be an exciting area of future research.

Why are studies on chymase of potential interest to the clinical nephrologist? Gone are the good old days when matters were simple and straightforward, and when it was thought that inhibition of the ACE blocked the transformation of AngI to AngII completely. If there are indeed important, ACE-independent pathways for the generation of AngII, as we suspect today, one would predict that Ang receptor inhibitors would be more potent than ACE inhibitors. Currently the evidence from cardiac studies does not unequivocally support the hypothesis of a greater potency of angiotensin receptor blockers. These negative findings are paradoxical, because the human heart strongly expresses chymase (31).

With respect to the kidney, direct head-on comparisons between ACE inhibitors and angiotensin receptor blockers are not available, but a further twist has recently been introduced by the demonstration in the COOPERATE study (32) that the combination of the maximally licensed doses of an ACE inhibitor and angiotensin receptor blocker provides greater renoprotection than the monotherapies. One of the potential explanations may well be that angiotensin receptor blockers interfere with pathways that are unaffected by ACE inhibitors.

Thus, the study of Huang et al., although not providing direct functional evidence for a role of chymase, provides another strong stimulus to address these unresolved questions by appropriate human studies.

References


