Chymase Is Upregulated in Diabetic Nephropathy: Implications for an Alternative Pathway of Angiotensin II–Mediated Diabetic Renal and Vascular Disease

XIAO R. HUANG,* WEI Y. CHEN,† LUAN D. TRUONG,* and HUI Y. LAN*
*Departments of Medicine-Nephrology and Pathology, Baylor College of Medicine, Houston, Texas; and
†Department of Nephrology, The First Hospital, Sun Yat-Sen University, Guangzhou, China.

Abstract. Angiotensin II (AngII) has been shown to play a critical role in diabetic nephropathy and vasculopathy. Although it is well recognized that an angiotensin-converting enzyme (ACE)–dependent AngII-generating system is a major source of intrarenal AngII production, it is here reported that the chymase-dependent AngII-generating system is upregulated in the human diabetic kidney. This becomes particularly strong in those with hypertension. In the normal kidney, while ACE was constitutively expressed by most kidney cells, chymase was weakly expressed by mesangial cells (MC) and vascular smooth muscle cells (VSMC) only. In the diabetic kidney, while ACE expression was significantly upregulated (1 to 3-fold) by tubular epithelial cells (TEC) and infiltrating mononuclear cells, there was also markedly increased chymase expression (10 to 15-fold) by both MC and VSMC, with strong deposition in the collagen-rich extracellular matrix including both diffuse and nodular glomerulosclerosis, tubulointerstitial fibrosis, and vascular sclerosis. Interestingly, while ACE expression showed no difference in patients with or without hypertension, upregulation of chymase in hypertensive patients was much stronger than that seen in those without hypertension (4 to 7-fold, $P < 0.001$). Correlation analysis showed that, in contrast to the ACE expression, upregulation of chymase correlated significantly with the increase in BP and the severity of collagen matrix deposition within the glomerulus, tubulointerstitium, and arterial walls (all with $P < 0.001$). In conclusion, the present study demonstrates that chymase, as an alternative AngII-generating enzyme, is markedly upregulated in the diabetic kidney and may be associated with the development of diabetic/hypertensive nephropathy. In addition, differential expression of ACE and chymase in the diabetic kidney indicates that both ACE and chymase may be of equal importance for AngII-mediated diabetic nephropathy and vascular disease. Results from this study suggest that blockade of both AngII-generating pathways may provide additional beneficial effect on diabetic nephropathy.

Diabetic nephropathy is a leading cause of end-stage renal disease. Experimental and clinical studies over the last decade have indicated that the intrarenal renin-angiotensin system (RAS), particularly the effector molecule angiotensin II (AngII), plays a pivotal role in diabetic nephropathy (1,2). This is supported by the findings that blockade of AngII with either angiotensin-converting enzyme (ACE) inhibitor or an angiotensin type 1 (AT1) receptor antagonist can prevent or delay the progression of renal injury associated with diabetes (3–6).

Glomerular mesangial cells (MC), tubular epithelial cells (TEC), and juxtaglomerular cells contain all of the components of the RAS, including renin, angiotensinogen (Atg), ACE, and AT1 and AT2 receptors, which are necessary for synthesis and secretion of AngII (7,8). It has also been shown that the intrarenal AngII contributes largely to AngII-dependent hypertension (7,8). The use of ACE inhibitors only partially reduces the local AngII production, indicating that non–ACE-dependent AngII pathways may contribute to the intrarenal AngII formation (8). However, the nature of these potential alternative pathways remains unclear.

The traditional view that AngII formation is solely dependent on ACE has been recently challenged. Increasing evidence has demonstrated that alternative pathways to the ACE exists for AngII generation in the heart, arteries, and kidney in human, monkey, dog, hamster, and rat (9,10). The most important of these alterative pathways in the cardiovascular system is thought to be chymase-dependent (9,10), as AngII formation is substantially blocked by chymase inhibitors such as chymostatin, NK3201, and CD41 (11–13). It has been shown that more than 80% of AngII formation in the human heart and more than 60% of that in arteries appears to be chymase-dependent (14). In chronic heart failure and coronary heart disease, both ACE-dependent and ACE-independent (chymase) pathways exist and full blockade of RAS requires both ACE and chymase inhibition (14). However, it remains completely unknown whether the chymase pathway is activated in diabetic nephropathy.

Recent study in ACE-knockout mice has shown that local

Received February 13, 2003. Accepted March 26, 2003.
Correspondence to Dr. Hui Y. Lan, Department of MedicineRenal Section, Associate Professor of Medicine, Baylor College of Medicine, One Baylor Plaza, Alkek N520, Houston, TX 77030. Phone: 713-798-1303; Fax: 713-798-5010; E-mail: hlan@bcm.tmc.edu
1046-6673/1407-1738
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000071512.93927.4E

AngII generation within the kidney is unchanged due to a 14-fold increase in the chymase activity (15). This finding allows us to hypothesize that chymase may involve the intrarenal AngII formation not only in physiologic but also in certain pathologic conditions such as diabetic nephropathy. In the present study, we tested this hypothesis in the human diabetic kidney by immunohistochemical staining with the anti-chymase monoclonal antibody and found that chymase was markedly upregulated in both MC and VSMC. These observations indicate that the chymase-dependent AngII-generating system may contribute to AngII-mediated glomerulosclerosis and vasculopathy in diabetes.

Materials and Methods

Kidney Tissues

Kidney tissues (12 autopsies and 32 biopsies) with unequivocal type II diabetic nephropathy were obtained from the Department of Pathology, Baylor College of Medicine. The study was approved by the University Review Board. The renal pathologic changes included diffuse glomerulosclerosis (13 cases), nodular glomerulosclerosis (31 cases), and moderate to severe tubulointerstitial fibrosis and vascular sclerosis (36 cases). All patients received a conventional anti-hypertension treatment such as calcium channel blockers and direct-acting vasodilators. Patients treated with either ACE inhibitors or AT1 receptor antagonists were excluded from this study. Clinical information including BP, blood glucose, and renal functional damage was summarized in Table 1. In addition, five normal kidney tissues from mismatched kidney donors (two cases) or from the non-neoplastic portion of the kidneys removed for renal carcinoma (three cases) were used as controls.

Immunohistochemistry

Four-micron sections from the formalin-fixed, paraffin-embedded kidney tissue were stained with mouse monoclonal antibodies to human chymase and ACE and rabbit polyclonal antibodies to collagen I and IV (Chemicon, Temecula, CA) using the microwave-based antigen retrieval technique and a modified peroxidase anti-peroxidase method as described previously (16). Briefly, after microwaving (10 min) in 0.01 M citric buffer (pH 6.0), sections were digested with 0.1% trypsin (Life Technologies BRL, Gaithersburg, MD) for 10 min at room temperature, followed by overnight incubation with antibodies to human chymase, ACE, collagen I, and collagen IV at 4°C. After inactivating endogenous peroxidase in 0.3% H_2O_2 in methanol, sections were labeled sequentially with rabbit anti-mouse IgG, mouse peroxidase-conjugated anti-peroxidase complexes (PAP), mouse peroxidase-conjugated Envision+ System (Dako Corporation, Carpinteria, CA), and developed with diaminobenzidine to produce a brown color.

In addition, double immunostaining was performed to detect collagen types I and IV produced by VSMC in renal arterial walls (16). Briefly, after collagen I and IV being labeled with rabbit polyclonal antibodies (Chemicon) using a three-layer PAP method and developed with DAB to give brown-color products, sections were microwaved and incubated sequentially with a mouse monoclonal antibody against α-smooth muscle actin (α-SMA, Dako), alkaline phosphatase-conjugated goat anti-mouse IgG, and mouse alkaline phosphatase anti-alkaline phosphatase complexes, and then developed with Fast Blue BB Salt (Ajax Chemicals, Melbourne, Australia) to produce a blue product. An irrelevant isotype mouse monoclonal antibody against rat CD45 (OX-1, IgG1) or rabbit IgG1 were used as negative controls. Sections were then mounted and examined under microscope.

Quantitative Analyses

Quantitative analyses of chymase and ACE expression and collagen accumulation in glomeruli, tubulointerstitium, and arterial walls were performed using quantitative Image Analysis System (Optima 6.5, Media Cybernatics, Silver Springs, MD). The percentages of glomerular, tubulointerstitial, and arterial wall areas with expression of chymase and ACE and deposition of collagen I and collagen IV were quantified under a ×40 power field microscope. Briefly, all glomeruli (5–12), tubulointerstitial areas (10 to 20 high-power fields), and arterial profiles (3–7) in each renal biopsy or 20 glomeruli, 20 high-power fields and 10 arterial profiles randomly chosen from autopsy kidney tissues were examined. First, the examined area of the glomerulus, tubulointerstitium, and arterial wall was outlined, the positive staining patterns were identified, and the percent positive area in the examined area was then measured. The Bowman space and arterial lumen space were excluded from the measurement. All examinations were performed blindly on coded slides.

Statistical Analyses

Data obtained from this study were expressed as mean ± SD and analyzed by one-way ANOVA or by unpaired *t* test using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA).

Results

Chymase and ACE Expression in the Normal Human Kidney

In the normal kidney, immunohistochemistry demonstrated that MC, VSMC, and TEC were the major sites of ACE expression (Figure 1a). In addition, podocytes, vascular endothelial cells, and some interstitial cells also weakly expressed ACE (Figure 1a). However, chymase was constitutively expressed only by MC and VSMC with occasional positive cells in perivascular and interstitial compartments (Figure 1b). Interestingly, while ACE was strongly expressed by TEC, particularly in the brush border, chymase was completely absent in TEC (Figure 1, a and b).

Table 1. Clinical data from hypertensive and normotensive diabetic patients

Diabetic Patients	Number	Age (yr)	Disease Course (yr)	Blood Glucose (mg/dl)	BP (mmHg)	Proteinuria (g/24 h)	Serum Creatinine (mg/dl)
Hypertensive	15	50.5 ± 14.1	11.8 ± 5.4	216 ± 101	167 ± 20/88 ± 8.2[c]	5.1 ± 2.2[b]	5.4 ± 3.6[a]
Normotensive	29	52.8 ± 11.8	12.0 ± 1.9	212 ± 117	137 ± 7.9/73 ± 11.2	2.8 ± 1.5	3.0 ± 1.4

Data represent the mean ±SD; [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ compared with normotensive diabetic patients.

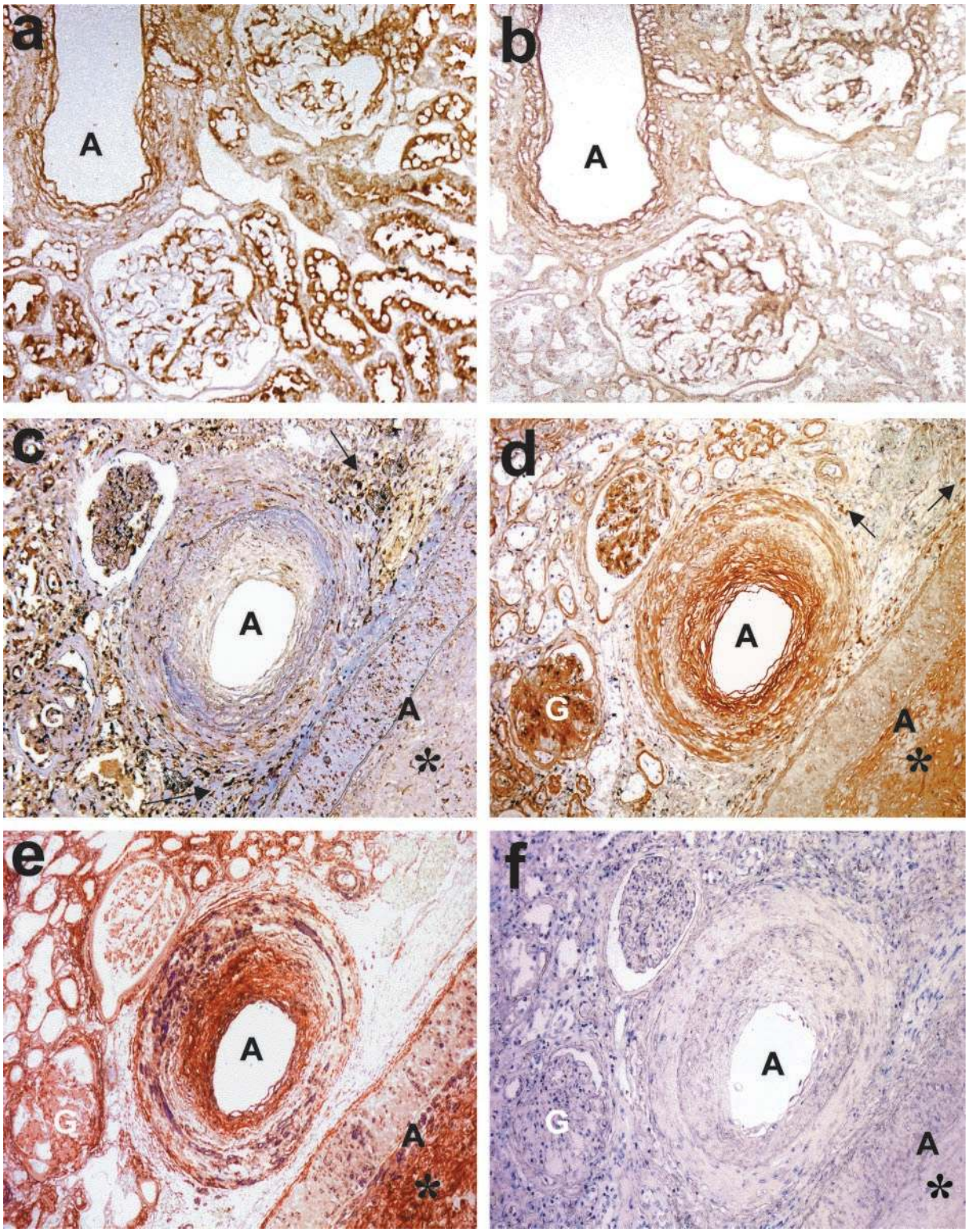

Figure 1. Angiotensin-converting enzyme (ACE) and chymase expression in normal and hypertensive diabetic nephropathy. (a and b) Immunohistochemistry demonstrates that in serial sections of a normal human kidney ACE is expressed by tubular epithelial cells (TEC), mesangial cells (MC), and vascular smooth muscle cells (VSMC) (a), while chymase is weakly expressed by MC and VSMC only (b). (c through f) Serial sections of a hypertensive diabetic kidney show that ACE is moderately upregulated in the damaged tubulointerstitium, but not in the sclerotic glomeruli and arteries (c). (d) In contrast, chymase is markedly upregulated by glomerular MC, VSMC, and proliferative intimal myofibroblasts as demonstrated by α-smooth muscle actin (α-SMA) expression (e*). Upregulation of chymase is tightly associated with glomerulosclerosis and arteriosclerosis, particularly with intimal thickening (*), as demonstrated by collagen I accumulation (e: blue staining = α-SMA; red = collagen I). (f) A serial section with an isotype control antibody (OX-1) staining, showing negative staining. A = arteries; arrows = immunoreative mononuclear cells. (*) intimal thickening. A = renal artery, G+ glomerulus. Magnification, $\times$200.

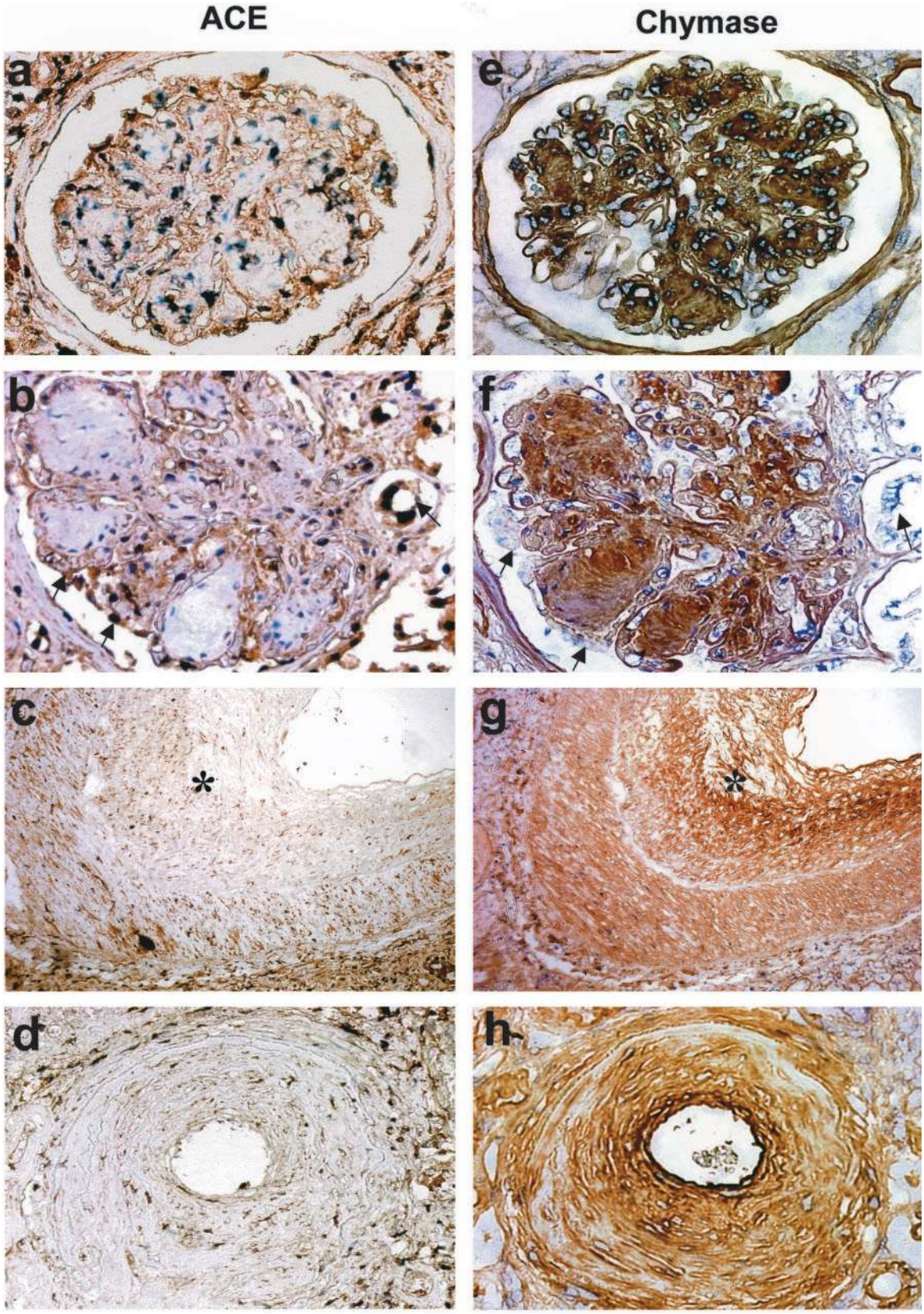

Figure 2. Comparison between chymase and ACE expression in hypertensive diabetic nephropathy. (a and e) A representative immunocytochemistry in serial sections shows that while ACE is upregulated by glomerular epithelial cells and mononuclear cells infiltrating the periglomerular area (a), chymase is markedly upregulated by glomerular MC with diffuse glomerulosclerosis (e). (b and f) Serial sections of a hypertensive diabetic kidney show that in contrast to ACE, which is upregulated by glomerular and tubular epithelial cells (b, arrows), chymase is strongly expressed by glomerular MC (f). Note that expression of chymase is absent in glomerular and tubular epithelial cells

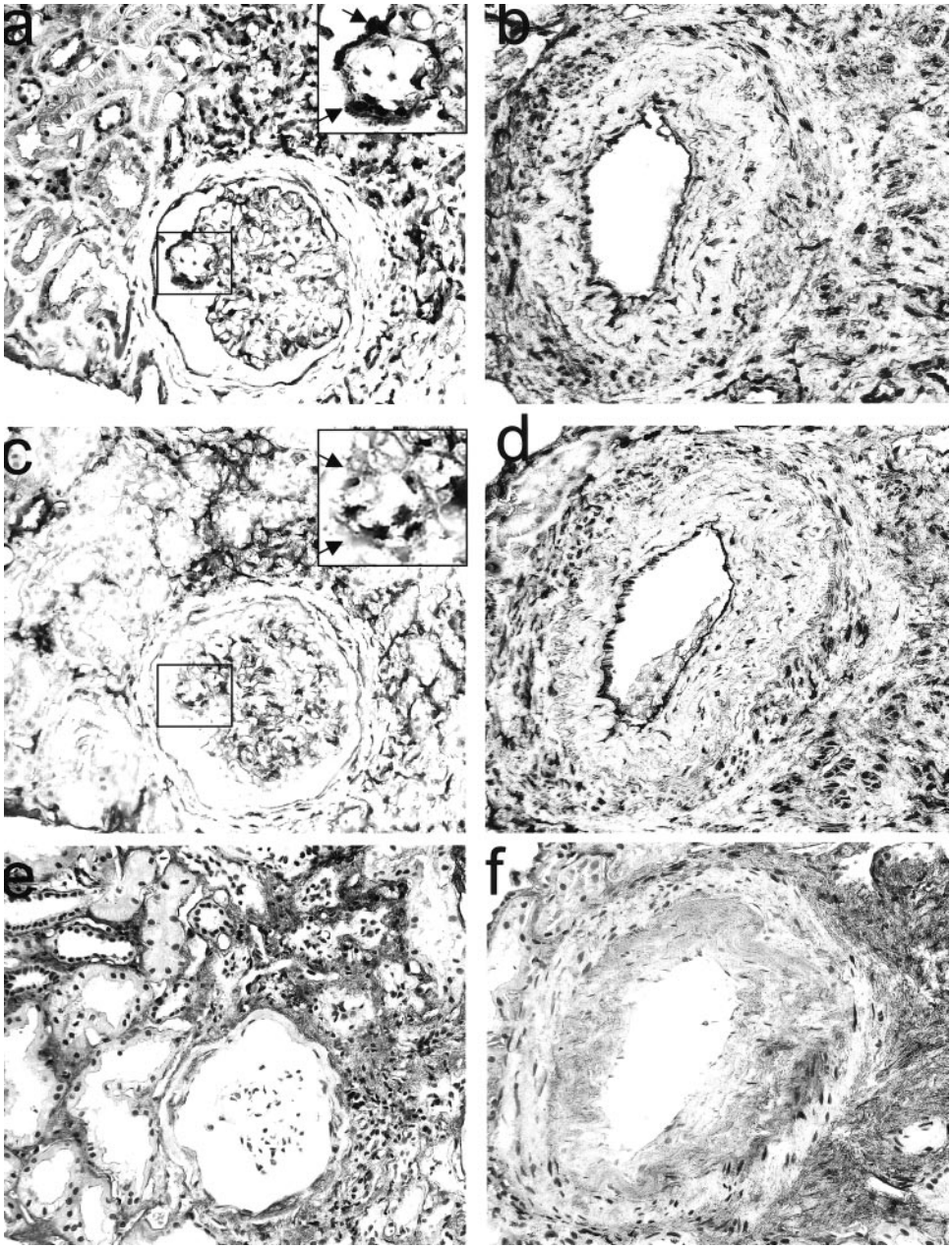

Figure 3. Comparison between chymase and ACE expression in a normotensive diabetic nephropathy. (a and c) A representative immunocytochemistry in serial sections shows that while ACE is upregulated by glomerular epithelial cells, TEC, and mononuclear cells infiltrating the periglomerular area (a), chymase is moderately upregulated by glomerular MC (e). This is clearly illustrated in a nodular lesion (see insert pictures, glomerular epithelial cells indicated by arrows). (b and d) Serial sections of a normotensive diabetic renal artery showing that both ACE (b) and chymase are moderately upregulated by VSMC and endothelial cells, but strongly expressed by perivascular myofibroblasts. (e and f) Collagen I immunostaining of serial sections to both ACE and chymase. Note that strong collagen I accumulation is co-localized to the areas with strong ACE and chymase expression in the periglomerular and perivascular regions. Magnification, ×250.

Figure 2.—Continued
(arrows), but accumulated in extracellular matrix-rich tissues such as nodular glomerulosclerosis and the thickening of Bowman and tubular basement membrane (e and f). (c and g) Serial sections of a renal artery with proliferative intimal thickening. Note that in contrast to ACE, which is moderately upregulated by medial VSMC but not by proliferative myofibroblasts in the area of intimal thickening (c, *), chymase is strongly expressed by VSMC, with more profound expression in the proliferative myofibroblasts in the region of intimal thickening (g, *). (d and h) Serial sections of a renal artery with advanced arteriosclerosis and abundant collagen matrix deposition. While ACE expression is absent (d), chymase remains strong (h). Magnification, ×400.

Chymase and ACE Expression in the Human Diabetic Kidney

In the kidneys with both hypertensive and diabetic changes, upregulation of ACE by both glomerular epithelial cells, TEC, and interstitial mononuclear cells and fibroblasts were remarkable, while upregulation of ACE by both MC and VSMC was only mild (Figures 1c and 2, a-d). A similar expression pattern of ACE was also found in the diabetic kidneys without hypertension (Figure 3, a and b).

In contrast, chymase was markedly expressed by MC and VSMC, but not by glomerular epithelial cells and TEC in the diabetic kidney with hypertension (Figure 1d and 2, e-h). Strong chymase immunoreactivity was also found in fibrotic tissues with rich collagen matrix accumulation, particularly in the mesangial region with diffuse or nodular sclerosis (Figures 1d and 2, e and f), vascular walls with arteriosclerosis (Figures 1d and 2, g and h), and thickened Bowman capsular and tubular basement membrane (Figures 1d and 2, e and f). In diabetic kidneys without hypertensive changes, upregulation of chymase by MC and VSMC remained significant (Figure 3, c and d) but was less strong than those with hypertensive changes (Figures 1d and 2, e-h). In addition, some chymase-positive cells were found in the interstitial and perivascular areas (Figures 1d and 3, c and d) in specimens with severe interstitial fibrosis (Figures 1e and 3, e and f).

Interestingly, there was differential expression between ACE and chymase within diabetic kidneys. This was further emphasized in sets of serial sections as shown in Figures 1 to 3. In contrast to ACE, which was significantly upregulated in glomerular and tubular epithelial cells and interstitial cells including infiltrating mononuclear cells and fibroblasts, chymase was strongly upregulated by MC (Figures 1c *versus* 1d, 2a *versus* 2e, 2c *versus* 2f, 3a *versus* 3c), VSMC and myofibroblasts in the areas of intimal thickening (Figures 1c *versus* 1d, 2c *versus* 2g, 2d *versus* 2h). Chymase was strongly accumulated in the sclerotic glomeruli including nodular sclerosis and the severe sclerotic arterial walls in which collagen matrix accumulation was abundant (Figures 1, d and e, and 2, e-h), but this was not seen in serial sections stained for ACE (Figures 1c and 2, a-d). These observations were further demonstrated by a quantitative analysis as shown in Figures 4 to 6.

The specificity of chymase and ACE staining within the kidney was demonstrated in Figure 1f, in which no staining was seen by the isotype control antibody, compared with both ACE and chymase staining in serial sections (Figure 1, c and d).

Correlation of Chymase and ACE Expression with Pathologic Changes in the Human Diabetic Kidney and Clinical Findings

We next investigated the relationship of chymase and ACE expression with clinical parameters such as BP, proteinuria, and serum creatinine and the severity of glomerulopathy and vasculopathy as determined by collagen matrix accumulation as described in Materials and Methods. As shown in Table 1, all patients developed moderate to severe renal injury. Compared with non-hypertensive diabetic patients, those with hypertension exhibited more severe renal injury as determined by a significant increase in urinary protein excretion and serum creatinine (Table 1). Interestingly, it is chymase expression, but not that of ACE, that correlated significantly with the

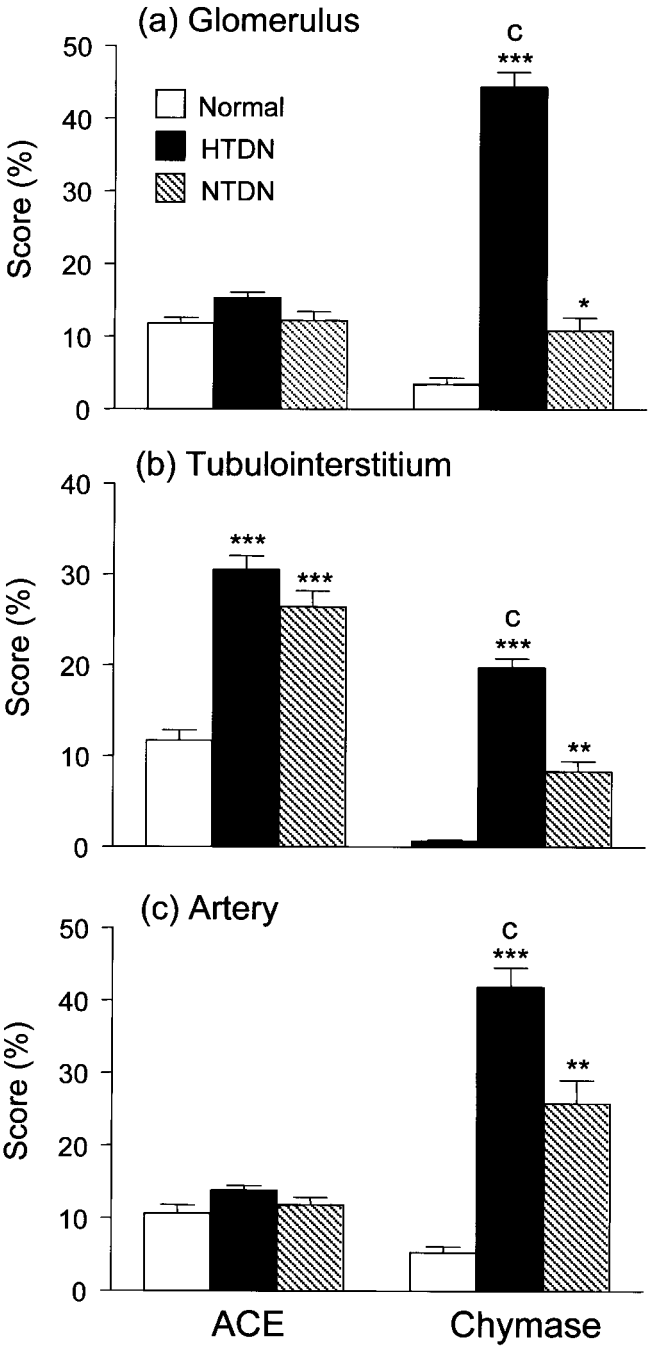

Figure 4. Quantitation of ACE and chymase expression in normal and diabetic nephropathy. ACE and chymase expression in glomeruli (a), tubulointerstitium (b), and arterial walls (c) are quantitated as described in Materials and Methods. Each bar represents the mean ± SEM for normal ($n = 5$), hypertensive ($n = 29$), and normotensive ($n = 15$) diabetic patients. HTDN, hypertensive diabetic nephropathy; NTDN, normotensive diabetic nephropathy. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with normal; $^{c}P < 0.001$ compared with normotensive diabetic patients.

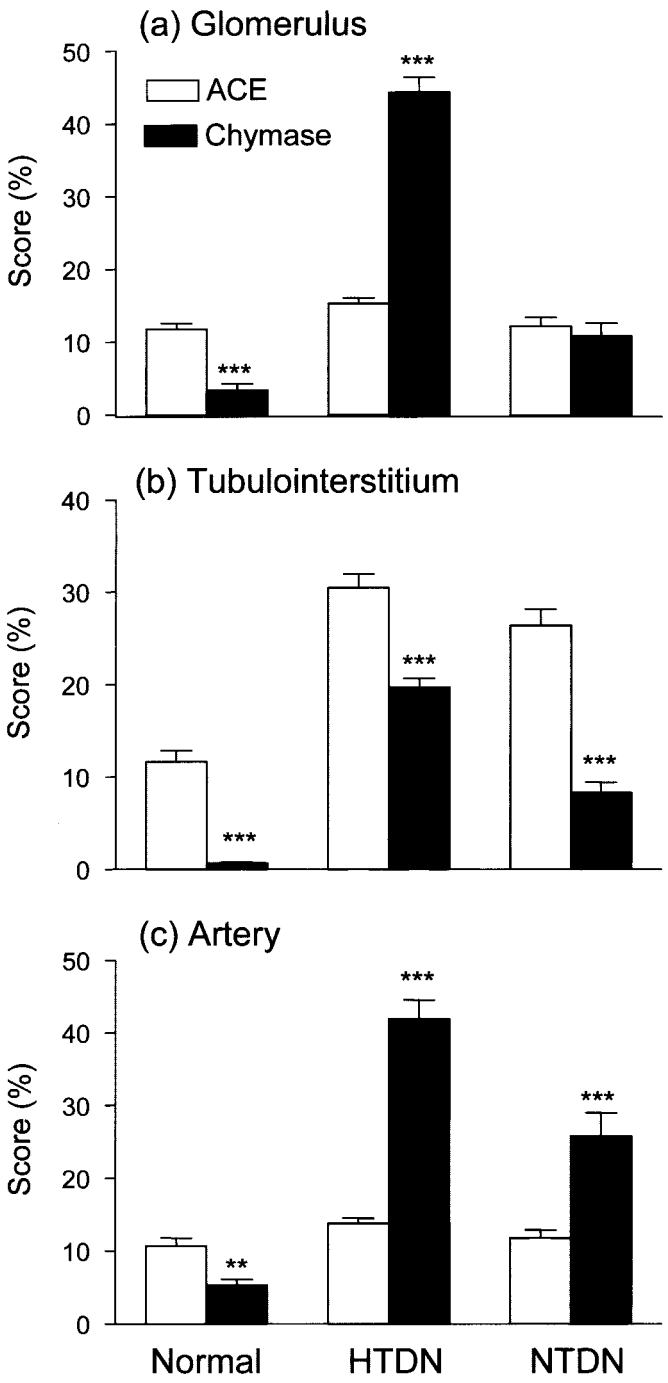

Figure 5. Comparison between ACE and chymase expression in normal, hypertensive, or normotensive diabetic nephropathy. ACE and chymase expression in glomeruli (a), tubulointerstitium (b), and arterial walls (c) are quantitated as described in the Method. Each bar represents the mean ± SEM for normal ($n = 5$), hypertensive ($n = 29$), and normotensive ($n = 15$) diabetic patients. HTDN, hypertensive diabetic nephropathy; NTDN, normotensive diabetic nephropathy. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with ACE.

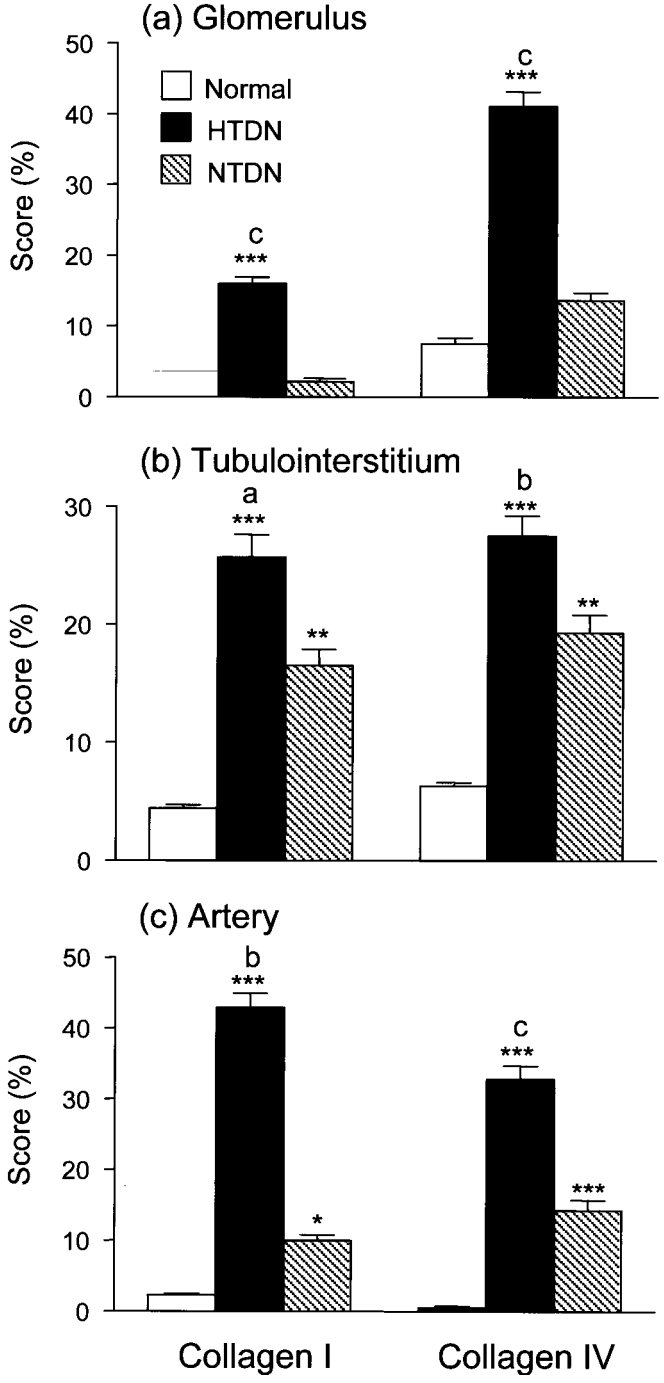

Figure 6. Quantitation of collagen I and collagen IV accumulation in normal and diabetic nephropathy. Collagen I and IV accumulation in glomeruli (a), tubulointerstitium (b), and arterial walls (c) are quantitated as described in Materials and Methods. Each bar represents the mean ± SEM for normal ($n = 5$), hypertensive ($n = 29$), and normotensive ($n = 15$) diabetic patients. HTDN, hypertensive diabetic nephropathy; NTDN, normotensive diabetic nephropathy. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with normal; $^{c}P < 0.001$ compared with normotensive diabetic patients.

increase in BP and proteinuria (all with $P < 0.05$). ACE expression, however, did correlate significantly with the increase in serum creatinine (Table 2). As shown in Figure 1, collagen matrix accumulation (Figure 1e) was exclusively associated with upregulation of chymase (Figure 1d), but not with ACE (Figure 1c), in glomerular mesangium, tubulointerstitium, and arterial walls, suggesting that upregulation of

Table 2. Correlation of ACE and chymase with clinical and pathological damage in diabetic nephropathy

	Tissue Sites	BP	Proteinuria	Serum Creatinine	Collagen I	Collagen IV
ACE	Glom	0.161	0.058	0.337[a]	0.140	0.183
	TI	0.064	0.135	0.106	0.040	0.076
	Artery	0.269	0.258	0.217	0.540[b]	0.40[a]
Chymase	Glom	0.492[b]	0.293	0.089	0.704[c]	0.632[c]
	TI	0.318[a]	0.380[a]	0.019	0.068	0.560[c]
	Artery	0.413[b]	0.310	0.080	0.580[c]	0.661[c]

Data represents a Pearson r value from 44 patients. BP, blood pressure; Glom, glomerulus; TI, tubulointerstitium. $n = 44$. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

chymase contributes to glomerulosclerosis, tubulointerstitial, and vascular fibrosis. It is further demonstrated in Table 2 that upregulation of chymase expression within the diabetic kidneys was highly correlated with the severity of collagen matrix accumulation within glomeruli, tubulointerstitium, and arterial walls (all with $P < 0.001$), while ACE expression correlated with collagen matrix deposition in arterial walls only ($P < 0.01$).

Discussion

A new and significant finding in this study is that chymase was strongly upregulated in diabetic kidneys. Upregulation of chymase is stronger in kidneys with hypertensive diabetic nephropathy than those with only diabetic nephropathy. Both MC and VSMC were the major sites of chymase expression. Upregulation of chymase by MC and VSMC contributed significantly to the development of diabetic glomerulosclerosis, tubulointerstitial fibrosis, and arterial sclerosis in terms of collagen matrix accumulation. In addition, upregulation of chymase also correlated significantly with the increase in BP and the severity of proteinuria. In contrast, increased ACE expression was found in glomerular epithelial cells and TEC as well as in infiltrating mononuclear cells, but it was NS in MC and VSMC. ACE expression within the diabetic kidneys did not correlate with glomerulosclerosis, tubulointerstitial fibrosis, and the increase in BP and proteinuria. However, increased ACE expression in tubulointerstitium did correlate with increase in serum creatinine. This may be associated with ACE-induced ischemic tubulointerstitial injury. Thus the present study demonstrates that both ACE-dependent and chymase-dependent AngII-generating systems are differentially upregulated in the diabetic kidney. It is likely that upregulation of chymase in glomerular MC and VSMC may be associated with AngII-mediated glomerulosclerosis and vascular sclerosis, although ACE may be also involved in AngII-mediated glomerular and vascular injury. Results obtained from this study suggest that chymase may be an alternative pathway for intrarenal angiotensin generation and may be associated with the development of diabetic/hypertensive nephropathy. However, it should be pointed out that because these observations are largely based on the descriptive and correlative data, results from this study could not demonstrate any functional differences between ACE and chymase expression in the development of hypertensive/diabetic nephropathy.

Chymases are serine proteinases that were originally thought to be produced by mast cells in blood vessels and the myocardium (17), but it was recently also found in rat VSMC (18). There are two forms of mammalian chymase, α and β, which differ in species and functions (9,10,19). Only α-chymase is found in human and baboons, while dogs, rats, and mice have both α- and β-chymases (9,10,19). Chymase has a maximal activity immediately upon release into the extracellular matrix after mast cells have been activated (11,20). In the human heart, chymase is synthesized and stored in mast cells, endothelial cells, and mesenchymal cells and is secreted directly to the interstitium, contributing up to 80% of AngII generation (17). In the present study, we found that abundant chymase was presented in the area of collagen-rich matrix accumulation such as nodular glomerulosclerosis, severe arteriosclerosis, and thickened tubular and Bowman's capsular basement membrane. This indicates that the extracellular chymase may represent an activated form of this enzyme. The functional importance of chymase was confirmed recently with the finding that conditional and targeted overexpression of rat vascular chymase causes hypertension in transgenic mice (21), which supports the present finding that upregulation of chymase is associated with the development of hypertension in diabetic patients. In addition, the successful prevention of intimal formation and restenosis after coronary angioplasty or catheter injury by an AT1 receptor antagonist, but not by an ACE inhibitor, also supports the critical role for the chymase-dependent pathway for AngII formation in vasculatures (22).

While the importance of chymase in cardiovascular diseases has been well documented, little is known about a role of chymase in diabetic nephropathy. In the human kidneys, increased expression of chymase in mast cells has been shown to correlate with the severity of interstitial fibrosis in the rejected kidney (23). However, little is known about chymase expression by the intrinsic kidney cells other than mast cells in both normal and disease kidney. By using a combination of microwave antigen retrieval method with a sensitive immunohistochemical technique, we are able to provide the evidence that chymase was constitutively expressed by MC and VSMC in the normal human kidney and was markedly upregulated in the

diabetic kidney. Consistent with the previous findings that upregulation of chymase is associated with cardiac fibrosis in the chronic stage of hypertension and renal fibrosis in rejected renal grafts (23,24), upregulation of chymase in diabetic kidneys contributed significantly to glomerulosclerosis, tubulointerstitial fibrosis, and arteriosclerosis. Furthermore, the identification of differential upregulation of ACE and chymase within the diabetic kidneys supports the notion that a therapy combining an AT_1 receptor blocker and ACE inhibitor appears to be an important approach for effectively decreased BP and/or preventing the progression of diabetic nephropathy in both experimental and human settings (25–29) as well as in other kidney diseases unrelated to hypertension or diabetes (30–35). Findings from the present study also provide a rationale for applying this combination regimen in the prevention and treatment of hypertensive and diabetic nephropathy.

In summary, the present study showed that, in addition to ACE, chymase is strongly upregulated in the diabetic kidneys, providing a direct evidence that chymase represents an alternative pathway for local AngII formation in the diabetic kidneys and may contribute to the progression of diabetic nephropathy. These findings suggest that blockade of both AngII-generating pathways with an ACE inhibitor and an AT1 receptor antagonist may provide additional benefit to patients with diabetes.

Acknowledgments

This work is supported by grants from the Juvenile Diabetes Research Foundation (JDRF 1-2001-596).

References

1. Cooper ME, Bonnet F, Oldfield M, Jandeleit-Dahm K: Mechanisms of diabetic vasculopathy: An overview. *Am J Hypertens* 14: 475–486, 2001
2. Leehey DJ, Singh AK, Alavi N, Singh R: Role of angiotensin II in diabetic nephropathy. *Kidney Int Suppl* 77: S93–S98, 2000
3. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD: The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 329: 1456–1462, 1993
4. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345: 851–860, 2001
5. Parving HH, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, Arner P: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345: 870–878, 2001
6. Brenner BM, Cooper ME, de Zeeuw DK, William FM, William E, Parving H-H, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 861–869, 2001
7. Siragy HM, Howell NL, Ragsdale NV, Carey RM: Renal interstitial fluid angiotensin. Modulation by anesthesia, epinephrine, sodium depletion, and renin inhibition. *Hypertension* 25: 1021–1024, 1995
8. Van Kats JP, Schalekamp MA, Verdouw PD, Duncker DJ, Danser AH: Intrarenal angiotensin II: Interstitial and cellular levels and site of production. *Kidney Int* 60: 2311–2317, 2001
9. Dell'Italia LJ, Husain A: Dissecting the role of chymase in angiotensin II formation and heart and blood vessel diseases. *Curr Opin Cardiol* 17: 374–379, 2002
10. Miyazaki M, Takai S: Local angiotensin II-generating system in vascular tissues: The roles of chymase. *Hypertens Res* 24: 189–193, 2001
11. Takai S, Yuda A, Jin D, Nishimoto M, Sakagichi M, Sasaki S, Miyazaki M: Inhibition of chymase reduces vascular proliferation in dog grafted veins. *FEBS Lett* 467: 141–144, 2000
12. Takai S, Jin D, Nishimoto M, Yuda A, Sakaguchi M, Kamoshita K, Ishida K, Sukenaga Y, Sasaki S, Miyazaki M: Oral administration of a specific chymase inhibitor. *NK* 3201: inhibits vascular proliferation in grafted vein. *Life Sci* 69: 1725–1732, 2001
13. Richard V, Hurel-Merle S, Scalbert E, Ferry G, Lallemand F, Bessou JP, Thuillez C: Functional evidence for a role of vascular chymase in the production of angiotensin II in isolated human arteries. *Circulation* 104: 750–752, 2001
14. Petrie MC, Padmanabhan N, McDonald JE, Hillier C, Connell JM, McMurray JJ: Angiotensin converting enzyme (ACE) and non-ACE dependent angiotensin II generation in resistance arteries from patients with heart failure and coronary heart disease. *J Am Coll Cardiol* 37: 1056–1061, 2001
15. Wei CC, Tian B, Perry G, Meng QC, Chen YF, Oparil S, Dell'Italia LJ. Differential ANG II generation in plasma and tissue of mice with decreased expression of the ACE gene. *Am J Physiol Heart Circ Physiol* 282: H2254–H2258, 2002
16. Lan HY, Mu W, Nickolic-Paterson DJ, Atkins RC: A novel, simple, reliable, and sensitive method for multiple immunoenzyme staining: Use of microwave oven heating to block antibody crossreactivity and retrieve antigens. *J Histochem Cytochem* 43: 97–102, 1995
17. Urata H, Boehm KD, Philip A: Cellular-localization and regional distribution of an angiotensin-II-forming chymase in the heart. *J Clin Invest* 91: 1269–1281, 1993
18. Cao ZM, Hulthen UL, Allen TJ, Cooper ME: Angiotensin converting enzyme inhibition and calcium antagonism attenuate streptozotocin-diabetes-associated mesenteric vascular hypertrophy independently of their hypotensive action. *J Hypertens* 16: 793–799, 1998
19. Okunishi H, Oka Y, Shiota N, Kawamoto T, Song K, Miyazaki M: Marked species-difference in the vascular angiotensin II-forming pathways: Human versus rodents. *Jpn J Pharmacol* 62: 207–210, 1993
20. Takai S, Shiota N, Kobayashi S, Matsumura E, Miyazaki M: Induction of chymase that forms angiotensin II in the monkey atherosclerotic aorta. *FEBS Lett* 412: 86–90, 1997
21. Ju H, Gros R, You X, Tsang S, Husain M, Rabinovitch M: Conditional and targeted overexpression of vascular chymase causes hypertension in transgenic mice. *Proc Natl Acad Sci USA* 98: 7469–7474, 2001
22. Miyazaki M, Wada T, Shiota N, Takai S: Effect of an angiotensin II receptor antagonist, candesartan cilexetil, on canine intima hyperplasia after balloon injury. *J Hum Hypertens* 12[suppl 1]: S21–S25, 1999
23. Yamada M, Ueda M, Naruko T, Tanabe S, Han YS, Ikura Y, Ogami M, Takai S, Miyazaki M: Mast cell chymase expression and mast cell phenotypes in human rejected kidneys. *Kidney Int* 59: 1374–1381, 2001

24. Shiota N, Jin D, Takai S, Kawamura T, Koyama M, Nakamura N, Miyazaki M: Chymase is activated in the hamster heart following ventricular fibrosis during the chronic stage of hypertension. *FEBS Lett* 406: 301–304, 1997
25. Cao Z, Bonnet F, Davis B, Allen TJ, Cooper ME: Additive hypotensive and anti-albuminuric effects of angiotensin-converting enzyme inhibition and angiotensin receptor antagonism in diabetic spontaneously hypertensive rats. *Clin Sci (Lond)* 100: 591–599, 2001
26. Wilkinson-Berka JL, Gibbs NJ, Cooper ME, Skinner SL, Kelly DJ: Renoprotective and anti-hypertensive effects of combined valsartan and perindopril in progressive diabetic nephropathy in the transgenic (mRen-2)27 rat. *Nephrol Dial Transplant* 16: 1343–1349, 2001
27. Mogensen CE, Neldam S, Tikkanen I, Oren S, Viskoper R, Watts RW, Cooper ME: Randomised controlled trial of dual blockade of renin-angiotensin system in patients with hypertension, microalbuminuria, and non-insulin dependent diabetes: The candesartan and lisinopril microalbuminuria (CALM) study. *BMJ* 321: 1440–1444, 2000
28. Hebert LA, Falkenhain ME, Nahman NS Jr, Cosio FG, O'Dorisio TM: Combination ACE inhibitor and angiotensin II receptor antagonist therapy in diabetic nephropathy. *Am J Nephrol* 19: 1–6, 1999
29. Kalender B, Ozturk M, Tuncdemir M, Uysal O, Dagistanli FK, Yegenaga I, Erek E: Renoprotective effects of valsartan and enalapril in STZ-induced diabetes in rats. *Acta Histochem* 104: 123–130, 2002
30. Russo D, Minutolo R, Pisani A, Esposito R, Signoriello G, Andreucci M, Balletta MM: Coadministration of losartan and enalapril exerts additive antiproteinuric effect in IgA nephropathy. *Am J Kidney Dis* 38: 18–25, 2001
31. Kincaid-Smith P, Fairley K, Packham D: Randomized controlled crossover study of the effect on proteinuria and blood pressure of adding an angiotensin II receptor antagonist to an angiotensin converting enzyme inhibitor in normotensive patients with chronic renal disease and proteinuria. *Nephrol Dial Transplant* 17: 597–601, 2002
32. Hilgers KF, Mann JFE: ACE Inhibitors versus AT1 receptor antagonists in patients with chronic renal disease. *J Am Soc Nephrol* 13: 1100–1108, 2002
33. Ferrari P, Marti HP, Pfister M, Frey FJ: Additive antiproteinuric effect of combined ACE inhibition and angiotensin II receptor blockade. *J Hypertens* 20: 125–130, 2002
34. Remuzzi A, Gagliardini E, Donadoni C, Fassi A, Sangalli F, Lepre SM, Remuzzi G, Benigni A: Effect of angiotensin II antagonism on the regression of kidney disease in the rat. *Kidney Int* 62: 885–894, 2002
35. Remuzzi G: ACE inhibitor and AT1 receptor antagonists: Is two better than one? *Kidney Int* 61: 1545–1547, 2002

See related editorial, "Chymase: A Potential Culprit in Diabetic Nephropathy," on pages 1952–1954.