CHAPTER 8

Antihypertensive therapy upregulates renin and (pro)renin-receptor in the clipped kidney of Goldblatt hypertensive rats

Christian Krebs
Inge Hamming
Salomeeh Sadaghiani
Oliver Steinmetz
Cristina Meyer-Schwesinger
Sabine Fehr
Rolf AK Stahl
Ingrid M Garrelds
Jan Danser
Harry van Goor
Aurelie Contrepas
Genevieve Nguyen
Ulrich Wenzel

Kidney International 2007;72:725–730

Abstract

Recently, a (pro)renin-receptor was identified which mediates profibrotic effects independently of angiotensin II. Antihypertensive therapy induces renal injury in the clipped kidney of 2 kidney, 1 clip hypertensive rats. This study examined the regulation of renin and (pro)renin-receptor in the clipped kidney of antihypertensively treated rats. Hypertensive Goldblatt rats (176±4 mmHg) were treated with increasing doses of the vasopeptidase inhibitor AVE 7688 (50, 150, 450 mg/kg food). Plasma prorenin and renin as well as renal renin and (pro)renin-receptor expression were studied.

The vasopeptidase inhibitor dose-dependently lowered blood pressure, which was associated with a massive upregulation of plasma prorenin and renin as well as increased renal expression of renin. In parallel with upregulation of renin, the (pro)renin-receptor was upregulated in the clipped kidney of Goldblatt rats. Immunohistochemistry demonstrated redistribution of renin upstream from the glomerulus in preglomerular vessels and renin staining in tubular cells. Expression of the (pro)renin-receptor was increased in vessels and tubules. This is the first observation of a parallel upregulation of renin and its receptor in vivo. As this upregulation was associated with thickening of renin positive vessels and tubulo-interstitial damage, these data suggests a profibrotic role for renin and the (pro)renin-receptor in the clipped kidney of antihypertensively treated Goldblatt rats.

Introduction

The renin angiotensin system is a central regulator of blood pressure. 70 years ago the landmark experiments of Goldblatt established that the system could also be responsible for disease[1]. Reduction of blood pressure by blockade of the renin angiotensin system has well-known and established beneficial effects in chronic kidney disease[2,3]. However, in case of renal artery stenosis, blood pressure reduction might have detrimental effects for the stenosed kidney due to renal failure. Prolonged blood pressure reduction in renovascular hypertension induces severe tubulointerstitial damage in the clipped kidney[4-7]. The mechanisms and mediators of this injury are poorly defined. However, it is known that in the clipped kidney renin levels are increased. From this point of view it is interesting that a (pro)renin-receptor was recently identified which binds prorenin and renin[8]. As both renin and prorenin are capable of binding with similar affinity, it was named (pro)renin-receptor. Upon receptor binding the enzymatic activity of renin is increased and prorenin is non-proteolytically activated. This indicates that the receptor may be involved in local angiotensin (Ang) II formation[9,10]. In addition, profibrotic signaling pathways are activated independent of Ang II[11]. Since it is unknown how the (pro)renin-receptor is regulated in vivo, especially in high renin conditions, we examined renin and (pro)renin receptor expression in the clipped kidney of Goldblatt rats. Moreover, the effects of blood pressure reduction by vasopeptidase inhibition on renin and (pro)renin-receptor[8] in the clipped kidney of Goldblatt hypertensive rats were studied.

Methods

Goldblatt hypertension

Studies were performed in male Sprague-Dawley rats (Charles River, Kisslegg, Germany). In rats weighing 120-140 g, 2 kidney, 1 clip hypertension was induced as described previously[5-7]. Only those rats with systolic blood pressure >160 mmHg 6 weeks after surgery were included in the protocol. 5 groups of animals were studied: normotensive control animals (n=12), hypertensive rats (n=16) and hypertensive rats treated for 6 weeks with threefold increasing doses of the vasopeptidase inhibitor AVE 7688 (kindly provided by Sanofi-Aventis, Frankfurt, Germany) (50 mg/kg (n=10), 150 mg/kg (n=11), 450 mg/kg food, (n=10)). The drug was provided with chow. Analyses were performed in three independent experiments with 3-5 animals per group. In addition, 4 normotensive rats were treated with AVE 450 mg/kg for 6 weeks and compared to 4 nontreated controls. The data on tubulointerstitial damage in this experiment have been published recently[7]. Systolic blood pressure was measured by tail cuff plethysmography (TSE-systems, Bad Homburg, Germany) in awake rats[6]. At the end of the experimental protocol blood was drawn from the aorta into ice-cold syringes containing heparin and the stenosed kidney was perfused with ice-cold phosphate buffered saline until the kidney blanched. The kidneys were removed and slices were fixed in 4% buffered formalin.

RNA, RT-PCR, in situ hybridization, histology and biochemical measurements

Total RNA from the cortex of the clipped kidney was prepared and real time RT-PCR performed as described previously [7]. The following rat specific PCR-primers were used in this study

Primer	Forward	reverse
renin	5'-GCT ACA TGG AGA ATG GGA CTG AA-3'	5'-ACC ACA TCT TGG CTG AGG AAA C-3'
(pro)renin-receptor	5'-TTC TGA ACT GCA AGT GCT GCA T-3'	5'-CTG CCA GCT CCA GTG AAT ACA AG-3'

The presented results are the means of 4 independent PCRs performed in duplicate with different RNAs pooled from 2-4 rats. In situ hybridization procedures were performed as described previously [7]. Sections were stained with periodic acid-Schiff (PAS) to evaluate renal morphology. Renin and (pro)renin-receptor immunohistochemistry were performed as described previously using polyclonal antibodies kindly provided by respectively Dr. T Inagami (Vanderbilt University School of Medicine, Nashville, USA) [Hamming] and Dr. G Ngyuen (INSERM, Paris)[8]. Alpha-smooth muscle cell actin staining was performed as described previously[7]. Planimetric examination of the renin positive preglomerular area was performed by means of of a Zeiss drawing tube in combination with a semiautomatic interactive image analysis system (Morphomat 30, Zeiss, Göttingen, Germany). The outlines of the renin positive preglomerular vessels were traced manually and the mean area was determined as described[6]. The number of renin positive tubular profiles divided by all tubular profiles was counted in 5 cortical high power fields in each kidney. The plasma levels of renin and prorenin were measured as described before. Prorenin was converted before measurement to renin by trypsin[26].

Statistical analysis

Results are expressed as means $\pm$ SEM. For multiple comparisons we used the Kruskal Wallis test with post hoc analysis according to Mann-Whitney-U. P values were adjusted for multiple testing to Bonferroni (statistical significance was defined as $P<0.05/k$ in case of k comparisons).

Results

Systolic blood pressure was increased in the Goldblatt rats and was dose-dependently reduced by the vasopeptidase inhibitor (Goldblatt rats 176±4 mmHg, Goldblatt+50 mg/kg AVE 168±8 mmHg, 150 mg/kg 133±7 mm Hg, 450 mg/kg AVE 94±4 mmHg). Blood pressure reduction by AVE induced a dose-dependent increase of interstitial fibrosis in the clipped kidney as recently shown[7]. Treatment of healthy normotensive rats with the vasopeptidase inhibitor (450 mg/kg) lowered blood pressure to 85±4 mmHg[7].

Plasma renin and prorenin concentrations were increased in untreated Goldblatt rats and increased further with reduction of blood pressure (figures 1A and 1B). The rise in plasma renin exceeded the rise in plasma prorenin, in agreement with the earlier observation that chronic stimulation causes more prorenin to be converted to renin, thereby leading to an increased renin/prorenin ratio in plasma[12].

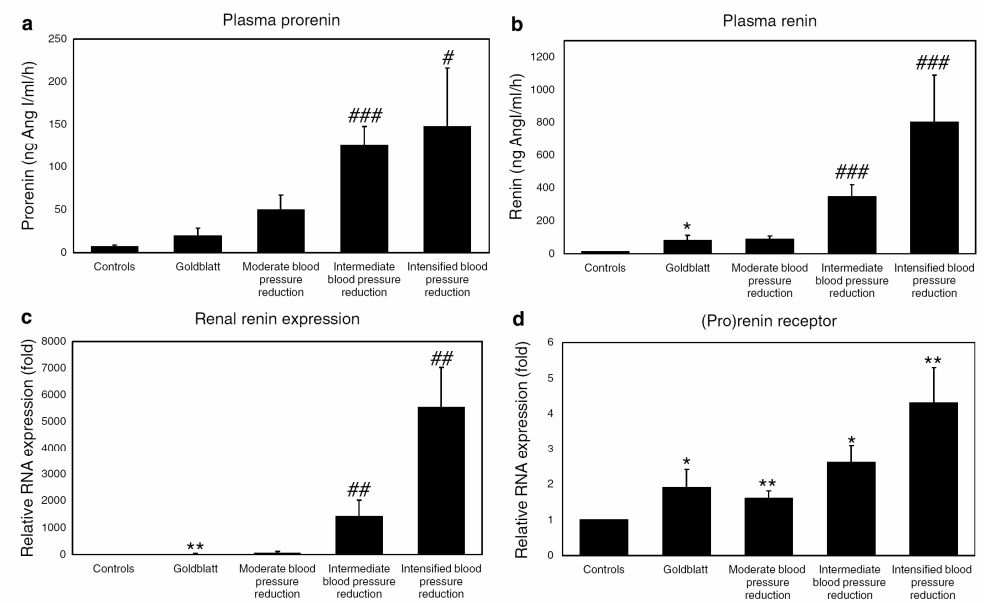

Figure 1. *Plasma prorenin and renin levels increased with blood pressure reduction* ***(A and B)****. Realtime RT-PCR analysis of kidney cortex revealed a dramatic upregulation of renin expression in the clipped kidney of antihypertensively treated rats* ***(C)****. The increased expression of renin was matched by an enhanced expression of the (pro)renin-receptor in treated and non-treated Goldblatt rats* ***(D)****. *P<0.007, **P<0.002 vs. controls, # P<0.012, ## P<0.004, ### P<0.001 vs. Goldblatt*

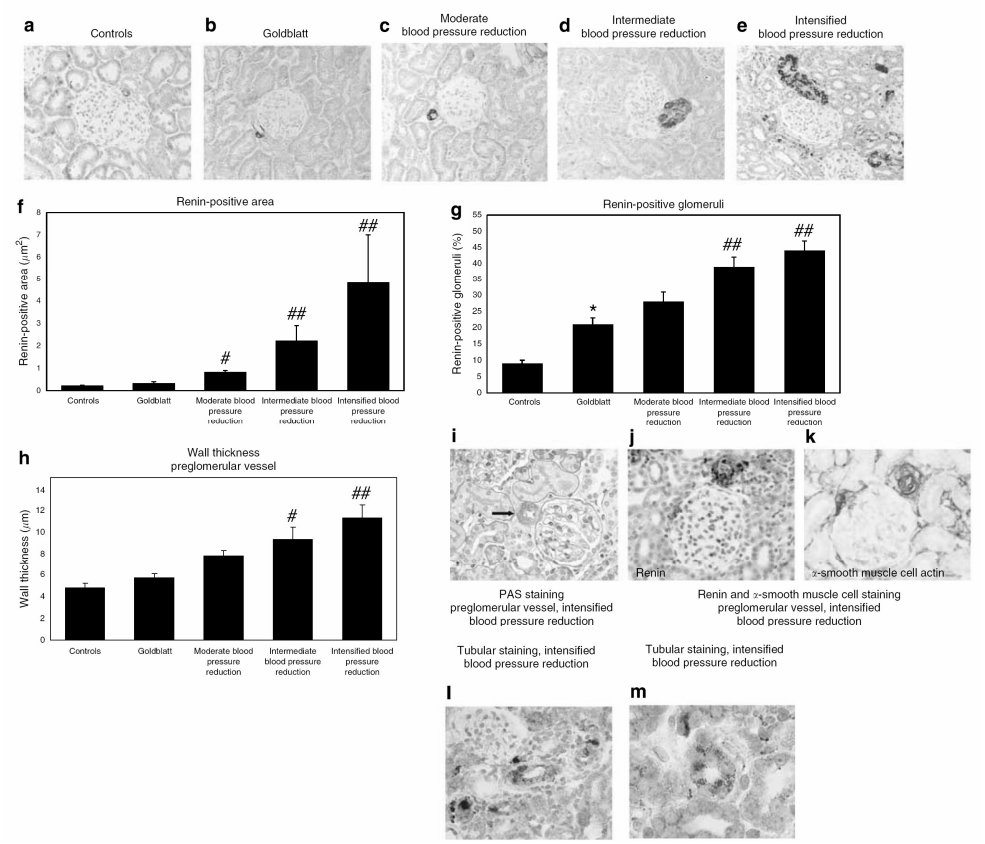

Figure 2. Immunohistochemical localization of renin protein. *Small parts of preglomerular vessels adjacent to the glomerulus stained positive for renin in controls and hypertensive rats* ***(A and B)****. With increasing blood pressure reduction a dose dependent increase of the renin positive area with a redistribution of renin upstream from the glomerulus in preglomerular vessels was found* ***(C-E)****. Figure* ***2F*** *shows a quantification of the renin positive area by morphometry. In addition the number of glomeruli with renin positive preglomerular vessels increased significantly with blood pressure reduction* ***(G)****. A significant increase in preglomerular vessel wall thickness was found in antihypertensively treated rats as shown in figure* ***2H*** *and* ***2I*** *(arrow). This is a combination of hyperplasia and conversion of smooth muscle cells into renin producing epithelial cells. Figure* ***2J*** *and* ***2K*** *show that the same preglomerular vessels stains positive for renin and alpha-smooth muscle cell actin on consecutive sections. In addition, renin staining was found in tubular cells of antihypertensively treated rats as shown in figure* ***2L*** *and* ***M****. *P<0.004 vs. controls #P<0.003, ##P<0.001 vs. Goldblatt*

Real-time RT-PCR showed that renal renin mRNA was upregulated in the clipped kidney of Goldblatt rats and that blood pressure reduction was associated with a massive dose-dependent upregulation of renin (figure 1C). In parallel to renin, (pro)renin-receptor mRNA was also upregulated in the clipped kidney of Goldblatt rats and antihypertensively treated rats (figure 1D). To study whether this effect is specific for the clipped kidney we evaluated the expression of renal renin and (pro)renin-receptor in healthy normotensive rats treated for 6 weeks with the vasopeptidase inhibitor (450 mg/kg). Blood pressure reduction induced a 92.5±7.4 fold increase of renal renin mRNA. However, (pro)renin-receptor mRNA was reduced 0.82±0.06 fold compared to untreated normotensive rats.

Immunohistochemistry against renin revealed an increasing redistribution of renin upstream from the glomerulus in preglomerular vessels of antihypertensively treated rats suggesting a recruitment of smooth muscle cells to the renin-expressing population (figure 2A-E). Dose dependent induction of renin was confirmed by morphometry of the renin positive area (figure 2F). Also the number of glomeruli with staining against renin of the preglomerular vessels increased significantly (figure 2G). An increased thickness of the wall of preglomerular vessels was observed in treated rats compared to untreated Goldblatt rats (figure 2H and I). In consecutive renal sections the same preglomerular vessel stained positive for renin and alpha-smooth muscle cell actin (figure 2 K and I). Some particular distal tubular cells of antihypertensively treated rats were positive for renin protein as well (figure 2L and M). 1 of 10 kidneys in the AVE 50 mg/kg, 2 of 11 in AVE 150 mg/kg and 5 of 10 in AVE 450 mg/kg group showed tubular renin staining. The number of renin-positive tubuli divided by all tubuli averaged 0.03±0.02 in the moderate, 0.02±0.01 in the intermediate and 0.15±0.08 in the intensified blood pressure reduction group. No renin positive tubuli were found in controls and Goldblatt rats.

In situ hybridization for renin showed weak preglomerular expression in controls (figure 3A and B), increased expression in Goldblatt rats and very strong renin expression in the antihypertensively treated Goldblatt rats (figure 3C and D). Tubular transcription of renin was not found in any group.

Weak (pro)renin-receptor mRNA expression was found in glomeruli, tubules and vessels in controls (figure 3E-G). Enhanced expression was seen in proximal and particularly in distal tubules and arteries of antihypertensively treated rats matching the results of the PCR data (figure 3H-J).

(Pro)renin-receptor protein is predominantly expressed in distal tubular cells and visceral epithelial cells of glomeruli (figure 4A). To a lesser extent (pro)renin-receptor is also found in proximal tubules and larger vessels. There was no co-localization of preglomerular renin and (pro)renin-receptor (figure 4A and B). However, the distal tubules that were positive for renin, also showed (pro)renin-receptor expression (figure 4C and D).

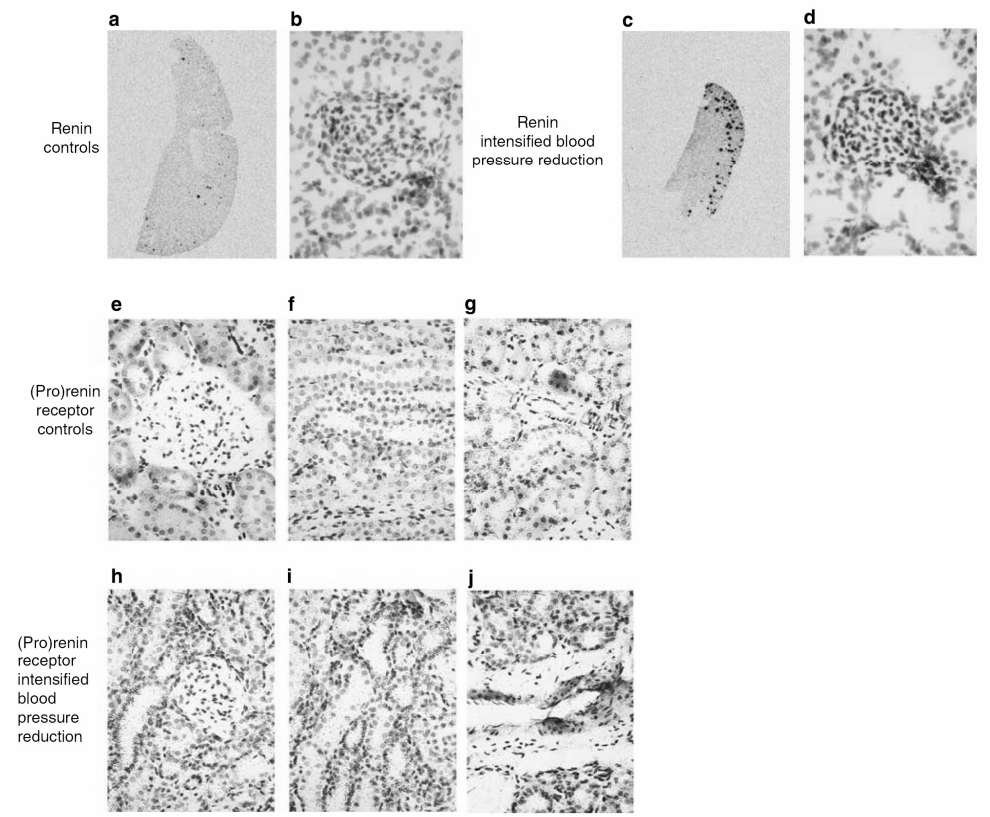

Figure 3. *In situ hybridization of renin showed weak expression in controls as shown in the autoradiograph of a whole kidney slice* ***(A)****. The weak preglomerular staining is shown in more detail in figure* ***3B****. In contrast, heavy staining of preglomerular vessels was found after blood pressure reduction as shown in the autoradiograph in figure* ***3C*** *and in more detail in figure* ***3D****. In situ hybridization of the (pro)renin- receptor revealed only weak glomerular, tubular and vessel staining in controls as shown in figure* ***3E-G****. In contrast enhanced staining was found in proximal and distal tubules as well as intrarenal vessels of antihypertensively treated rats as demonstrated in figure* ***3H-J****.*

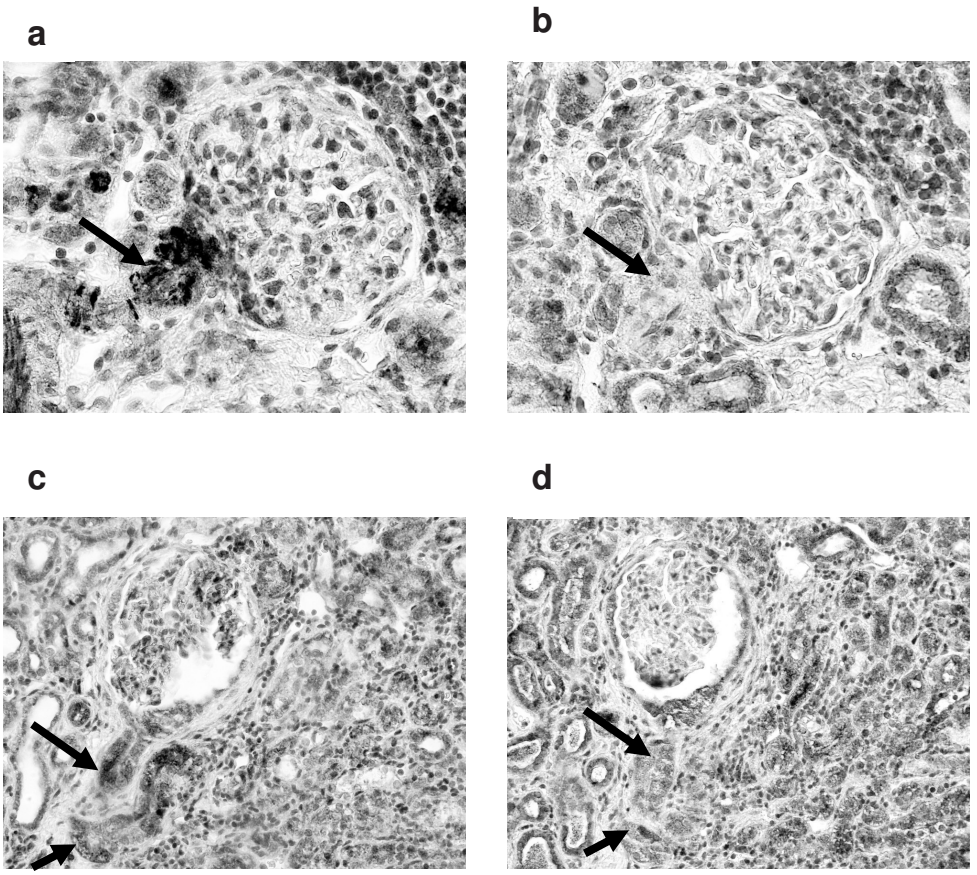

Figure 4. *Immunohistochemistry on consecutive sections for renal (pro)renin-receptor* ***(A and C)*** *and renin* ***(B and D)*** *in antihypertensively treated Goldblatt rats. The (pro)renin-receptor is not expressed in the preglomerular vessel* ***(A)*** *(arrow) which are renin positive* ***(B)*** *(arrow). In contrast, in tubuli (pro)renin- receptor expression and renin expression can be found on consecutive sections* ***(C and D)*** *(arrows).*

Discussion

Deleterious effects of blood pressure reduction can occur in patients with reduced renal perfusion due to stenosis of the renal artery or due to arteriosclerotic stenosis of intrarenal small vessels[13]. We and others have described the tubulointerstitial damage induced in these kidneys by antihypertensive therapy[4,6,14]. However, little is known about the mechanisms responsible for this pronounced injury in the clipped kidney.

In this study we showed that the renal renin and the (pro)renin-receptor are upregulated in parallel in the clipped kidney of Goldblatt hypertensive rats. Moreover, blood pressure reduction induced a further increase in renal renin expression in the clipped kidney. The in vivo regulation of the (pro)renin-receptor in the kidney is largely unknown. In healthy rats, upregulation of renal

renin along with a downregulation of (pro)renin-receptor was found during ACE inhibition and volume depletion[15]. This is in agreement with a recent observation that activation of the (pro)renin-receptor by renin results in translocation of the transcription factor promyelocytic zinc finger protein to the nucleus which subsequently represses transcription of the (pro)renin-receptor itself[16]. Since lowering of blood pressure in healthy rats also induced renal renin expression without upregulation of the (pro)renin-receptor expression, the parallel increments in the clipped kidney are apparently specific for Goldblatt hypertension and not a common regulatory mechanism.

Antihypertensive therapy induces renin expression in the clipped kidney. Renin protein was predominantly expressed in preglomerular vessels, but also weakly in tubules, which has been described previously[17]. The renin positivity of the preglomerular vessels may be due to conversion of vascular smooth muscle cells into renin producing epithelial cells. In consecutive section the preglomerular vessels stained positive for renin and alpha-smooth muscle cell actin. However, for a definitive proof this issue needs further investigation i. e. by dual label experiments with vascular and epithelial markers. As renin transcription was only found in preglomerular vessels, tubular renin is therefore probably derived from peritubular capillaries or the ultrafiltrate. Reabsorbed renin may bind to the (pro)renin-receptor, which was specifically upregulated in tubules in Goldblatt rats. Moreover, in tubules renin co-localizes with the (pro)renin-receptor and may upon binding cause tubulointerstitial injury through enhanced Ang II formation, which is known to initiate fibrotic pathways. However, blockade of ACE as done in the present study by using the vasopeptidase inhibitor, will suppress the generation of deleterious Ang II[18-20]. ACE is the predominant, if not the only, Angiotensin-converting enzyme in the kidney[18,19,21] and the bulk of renal Ang II is produced locally at renal tissue sites[20]. Therefore, the question arises, how fibrosis is mediated in the face of reduced renal Ang II generation. A plausible candidate is the recently cloned (pro)renin-receptor. Huang et al. recently showed that signaling of the (pro)renin-receptor after binding of renin has profibrotic effects independent of Ang II formation in vitro[1,11]. Furthermore, data by Ichihara suggest that binding of prorenin to the (pro)renin-receptor causes damage in hypertensive and diabetic renal injury also independent of Ang II[22,23]. This direct prorenin-induced effect involved activation of three members of the MAP kinase family, i.e. p38, ERK1/2 and Jnk. A similar renin/(pro)renin-induced activation of p38 and ERK1/2 MAP kinases has been proposed in mesangial cells and cardiomyocytes[8,24]. We therefore hypothesize that part of the renal injury in the clipped kidney after antihypertensive therapy may be caused by profibrotic signaling of the (pro)renin-receptor after binding of its ligands prorenin or renin.

The ultimate proof that the (pro)renin-receptor has profibrotic effects in the clipped kidney should be derived from the effects of blood pressure lowering in Goldblatt hypertensive (pro)renin receptor knockout mice. However, the ablation of the (pro)renin-receptor in embryonic stem cells is not compatible with their participation in embryonic development after injection into

blastocysts[25]. Unfortunately, the renin inhibitor aliskiren is also not a promising option as this drug is unlikely to interfere with renin binding to the (pro)renin-receptor[9,24].

In summary, our data provide the first observation of an upregulation of the (pro)renin-receptor in the clipped kidney of Goldblatt hypertensive rats. Blood pressure reduction induces a several thousand fold induction of renin and no downregulation of its receptor. The dose dependent increase in renin expression and the dose dependent increases of vascular and tubulo-interstitial injury strongly suggest a profibrotic role for the (pro)renin-receptor in the clipped kidney. This clearly suggests that interventions targeting renin or the (pro)renin-receptor merit further evaluation for the prevention or attenuation of renal failure in renovascular hypertension.

Acknowledgement

We thank S. Schröder, A. Peters and M. Rezka for excellent technical assistance.

References

1. Oliver JA: Receptor-mediated actions of renin and prorenin. Kidney Int 69:13-15, 2006

2. Wenzel UO: Angiotensin-converting enzyme inhibitors and progression of renal disease: evidence from clinical studies. Contrib Nephrol:200-211, 2001

3. Wolf G, Butzmann U, Wenzel UO: The renin-angiotensin system and progression of renal disease: from hemodynamics to cell biology. Nephron Physiol 93:P3-13, 2003

4. Jackson B, Franze L, Sumithran E, Johnston CI: Pharmacologic nephrectomy with chronic angiotensin converting enzyme inhibitor treatment in renovascular hypertension in the rat. J Lab Clin Med 115:21-27, 1990

5. Wenzel UO, Troschau G, Schoeppe W, Helmchen U, Schwietzer G: Adverse effect of the calcium channel blocker nitrendipine on nephrosclerosis in rats with renovascular hypertension. Hypertension 20:233-241, 1992

6. Wenzel UO, Wolf G, Jacob I, Schwegler C, Qasqas A, Amann K, Helmchen U, Stahl RA: Beneficial and adverse renal and vascular effects of the vasopeptidase inhibitor omapatrilat in renovascular hypertensive rats. Nephrol Dial Transplant 18:2005-2013, 2003

7. Steinmetz OM, Sadaghiani S, Panzer U, Krebs C, Meyer-Schwesinger C, Streichert T, Fehr S, Hamming I, van Goor H, Stahl RA, Wenzel U: Antihypertensive therapy induces compartment-specific chemokine expression and a Th1 immune response in the clipped kidney of Goldblatt hypertensive rats. Am J Physiol Renal Physiol 292:F876-887, 2007

8. Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD: Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. J Clin Invest 109:1417-1427, 2002

9. Danser AH: Prorenin: back into the arena. Hypertension 47:824-826, 2006

10. Saris JJ, van den Eijnden MM, Lamers JM, Saxena PR, Schalekamp MA, Danser AH: Prorenin-induced myocyte proliferation: no role for intracellular angiotensin II. Hypertension 39:573-577, 2002

11. Huang Y, Wongamorntham S, Kasting J, McQuillan D, Owens RT, Yu L, Noble NA, Border W: Renin increases mesangial cell transforming growth factor-beta1 and matrix proteins through receptor-mediated, angiotensin II-independent mechanisms. Kidney Int 69:105-113, 2006

12. Danser AH, Deinum J: Renin, prorenin and the putative (pro)renin receptor. Hypertension 46:1069-76, 2005

13. Toto RD, Mitchell HC, Lee HC, Milam C, Pettinger WA: Reversible renal insufficiency due to angiotensin converting enzyme inhibitors in hypertensive nephrosclerosis. Ann Intern Med 115:513-519, 1991

14. Rodriguez-Iturbe B, Quiroz Y, Kim CH, Vaziri ND: Hypertension induced by aortic coarctation above the renal arteries is associated with immune cell infiltration of the kidneys. Am J Hypertens 18:1449-1456, 2005

15. Hamming I, Contrepas A, Nguyen G, Huitema S, Navis GJ, van Goor H: Regulation of the renal renin/(pro)renin receptor by ACE-inhibition and sodium restriction. J Am Soc Nephrol 16:120:Abstract, 2005

16. Schefe JH, Menk M, Reinemund J, Effertz K, Hobbs RM, Pandolfi PP, Ruiz P, Unger T, Funke-Kaiser H: A novel signal transduction cascade involving direct physical interaction of the renin/prorenin receptor with the transcription factor promyelocytic zinc finger protein. Circ Res 99:1355-1366, 2006

17. Prieto-Carrasquero MC, Harrison-Bernard LM, Kobori H, Ozawa Y, Hering-Smith KS, Hamm LL, Navar LG: Enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. Hypertension 44:223-229, 2004

18. Schalekamp MA, Danser AH: Angiotensin II production and distribution in the kidney: I. A kinetic model. Kidney Int 69:1543-1552, 2006

19. Schalekamp MA, Danser AH: Angiotensin II production and distribution in the kidney: II. Model-based analysis of experimental data. Kidney Int 69:1553-1557, 2006

20. 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

21. Campbell DJ, Alexiou T, Xiao HD, Fuchs S, McKinley MJ, Corvol P, Bernstein KE: Effect of reduced angiotensin-converting enzyme gene expression and angiotensin-converting enzyme inhibition on angiotensin and bradykinin peptide levels in mice. Hypertension 43:854-859, 2004

22. Ichihara A, Kaneshiro Y, Takemitsu T, Sakoda M, Nakagawa T, Nishiyama A, Kawachi H, Shimizu F, Inagami T: Contribution of nonproteolytically activated prorenin in glomeruli to hypertensive renal damage. J Am Soc Nephrol 17:2495-2503, 2006

23. Ichihara A, Suzuki F, Nakagawa T, Kaneshiro Y, Takemitsu T, Sakoda M, Nabi AH, Nishiyama A, Sugaya T, Hayashi M, Inagami T: Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. J Am Soc Nephrol 17:1950-1961, 2006

24. Saris JJ, t Hoen PA, Garrelds IM, Dekkers DH, den Dunnen JT, Lamers JM, Jan Danser AH: Prorenin induces intracellular signaling in cardiomyocytes independently of angiotensin II. Hypertension 48:564-571, 2006

25. Nguyen G: Renin/prorenin receptors. Kidney Int 69:1503-1506, 2006

26. de Lannoy LM, Danser AH, van Kats JP, Schoemaker RG, Saxena PR, Schalekamp MA: Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart. Local production of angiotensin I. Hypertension 29:1240-1251, 1997