(Pro)Renin Receptor Peptide Inhibitor “Handle-Region”
Peptide Does Not Affect Hypertensive Nephrosclerosis in Goldblatt Rats

Dominik N. Muller, Bernd Klanke, Sandra Feldt, Nada Cordasic, Andrea Hartner, Roland E. Schmieder, Friedrich C. Luft, Karl F. Hilgers

Abstract—The (pro)renin receptor [(P)RR], a new component the renin-angiotensin system, was cloned recently. The (P)RR promotes direct mitogen-activated protein kinase signaling and nonproteolytic prorenin activation. We investigated the role of a (P)RR blocker, a peptide consisting of 10 amino acids from the prorenin prosegment called the “handle-region” peptide (HRP), on target organ damage in renovascular hypertensive 2-kidney, 1-clip (2K1C) rats. Vehicle-treated 2K1C rats were compared with HRP-treated 2K1C rats (3.5 μg/kg per day) and sham-operated controls. Vehicle-treated 2K1C rats developed hypertension (186±17 mm Hg), cardiac hypertrophy (3.16±0.16 mg/g), renal inflammation, fibrosis, vascular, and tubular damage. Chronic HRP treatment did not affect blood pressure (194±15 mm Hg), cardiac hypertrophy (2.97±0.11 mg/g), or renal damage. Furthermore, we investigated the renal renin and (P)RR expression. The clipped kidney of 2K1C and HRP-treated 2K1C rats showed a higher renin expression and juxtaglomerular index compared with sham-operated kidneys. The unclipped kidney showed suppressed renin expression. In contrast, (P)RR mRNA expression was not altered in any group. Plasma renin activity and aldosterone were increased in 2K1C rats compared with sham controls. HRP-treated 2K1C rats tended to lower plasma renin activity but showed similar aldosterone levels as vehicle-treated 2K1C rats. Our results indicate that blockade of the (P)RR with HRP does not improve target organ damage in renovascular hypertensive rats. (Hypertension. 2008;51:1-6.)

Key Words: renin ▪ (pro)renin receptor ▪ HRP ▪ target organ damage ▪ angiotensin ▪ renovascular hypertension

Nguyen et al1 cloned a novel (pro)renin receptor [(P)RR], a single transmembrane-domain protein of 350 amino acids with a large unglycosylated and highly hydrophobic N-terminal domain and a short cytoplasmic tail of ≈20 amino acids that interacts with both renin and prorenin. The (P)RR is highly conserved across species.2 The (P)RR binds renin and prorenin. When renin is bound to the (P)RR, the protein initiates extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase activation that is independent of angiotensin (Ang) II.1,3,4 Furthermore, renin bound to the (P)RR displays a 3- to 5-fold increased catalytic activity compared with renin in solution.1,5 Prorenin, which normally shows no catalytic activity in solution, is nonproteolytically activated when bound to the (P)RR.1,5 Suzuki et al6 investigated the mechanism of nonproteolytical activation of prorenin. They identified 2 regions in the prorenin segment, namely, T7PFKR10P and I11PFKLKR15P. These regions are crucial for nonproteolytic activation. Ichihara et al7 have synthesized a decoy peptide corresponding with the structure of this handle region (handle-region peptide [HRP]) and assumed that HRP must competitively bind to (P)RR as a decoy peptide, thereby inhibiting the nonproteolytic activation of prorenin.

The group provided numerous remarkable studies demonstrating that HRP treatment in diabetic mice and rats improved nephropathy without affecting blood glucose levels.7–9 The group also showed that HRP ameliorated renal and cardiac damage in hypertensive spontaneously hypertensive rats.10,11 Furthermore, the group generated a transgenic rat model that overexpressed the human (P)RR. The transgenic rats manifested proteinuria and glomerulosclerosis with aging or without increasing renal Ang II levels. HRP treatment suppressed the development of proteinuria and glomerulosclerosis without changing renal Ang II levels, whereas an Ang-converting enzyme inhibitor treatment was not renoprotective, despite a reduced renal Ang II level.12 These intriguing results prompted us to test the hypothesis that HRP treatment ameliorates the progression of target-organ damage in renovascular Goldblatt hypertensive rats. In our rat model of Goldblatt hypertension, blood pressure rises during the

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From the Medical Faculty of the Charité (D.N.M., S.F., F.C.L.), Experimental and Clinical Research Center, Franz Vollhard Clinic, and HELIOS Klinikum Berlin-Buch and Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany; and the Department of Nephrology and Hypertension (B.K., N.C., R.E.S., K.F.H.) and Childrens’ Hospital (A.H.), University of Erlangen-Nuremberg, Erlangen, Germany.

Correspondence to Karl F. Hilgers, Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Loschgestrasse 8, 91054 Erlangen, Germany. E-mail karl.hilgers@rzmail.uni-erlangen.de

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first 2 weeks after placement of the clip on the left renal artery; marked kidney damage develops in the next 2 weeks.13,14 We infused HRP from day 14 to day 28 after clipping.

Methods

Renovascular Hypertension

Rats were housed in a room maintained at 22±2°C, exposed to a 12 hour light/dark cycle. The animals were allowed unlimited access to chow (1320, Altromin) and tap water. All of the procedures performed on animals were done in accordance with the National Institutes of Health guidelines and were approved by the local government authorities. Two-kidney, 1-clip (2K1C) renovascular hypertension was induced in male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 150 to 170 g by placing a silver clip of 0.2-mm ID around the left renal artery through a flank incision under isoflurane anesthesia, as described previously.14 Control animals underwent sham operation without placement of the clip. Weight and systolic blood pressure (by tail cuff plethysmography) were measured weekly.

Treatment

After 2 weeks, only animals with systolic blood pressure >150 mm Hg were included in the 2K1C groups, which was achieved in 80% to 90% of all of the operated animals. To block the (P)RR, rats were treated with rat HRP (NH2-RILLKKMPSV-COOH, Biosynth), Seven 2K1C rats received rat HRP (3.6 μg/kg per day, SC), whereas 8 2K1C rats and 5 sham-operated rats received vehicle. Osmotic minipumps (Alzet model 2002, Alza), which delivered 0.5 μL/h for 14 days, were implanted SC under isoflurane anesthesia. Our HRP dose corresponded with 0.1 mg/kg per 28 days, the dose that was used in a previous study.15,16 We infused HRP from day 14 to day 28 after clipping.

Measurement of Plasma Renin and Aldosterone

Blood for analysis was collected from indwelling catheters. Thereafter, rats were killed by an overdose of thiopental. Plasma renin activity was assessed by determination of the conversion of angiotensinogen to Ang I. Ang I was measured by radioimmunoassay after incubation at 37°C for 1 hour, as described previously.14 Plasma aldosterone was measured by a commercially available radioimmunoassay kit (Aldosterone Maia 12254, Serono Diagnostics).

Immunohistochemistry

After organ weighing, kidneys were decapsulated. Part of each kidney was immediately snap frozen on liquid nitrogen for protein and RNA extraction, whereas a second part was put in methyl-Carnoy solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) for fixation. After overnight fixation, tissues were dehydrated and embedded in paraffin. Two-μm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments). After deparaffinization and blockade of endogenous peroxidase activity, the immunohistochemical detection of renin (rabbit antiserum kindly provided by Dr. Walter Fischli, Basel, Switzerland), macrophages (monoclonal ED-1 antibody, Biozol), and collagen I (Biozol), respectively, was performed as described previously.15,16 The Vectastain dianaminobenzidine kit (Vector Laboratory) was used as a chromogen. Each slide was counterstained with hematoxylin. Renal interstitial macrophages were counted as described previously in 30 medium-power (magnification: ×250) cortical views per section and expressed as cells per square millimeter.16 Interstitial collagen I was expressed as cells per square millimeter.16 Interstitial collagen I was expressed as a percentage of the total number of glomeruli counted. These percentage values were used for statistical analysis.

Statistical Analysis

We relied on 2-way ANOVA, followed by the least significant difference posthoc test, to test significance of differences between groups. A P value <0.05 was considered significant. Calculations were carried out using the SPSS 15.0 software (SPSS Inc).

Results

During the course of the experiment, 1 2K1C rat receiving vehicle and 2 2K1C rats receiving HRP died, whereas all of the sham-operated animals survived. The body weight of 2K1C rats tended to be lower than that of sham-operated animals, but there was no significant difference (Table). Systolic blood pressure continued to increase after implantation of the minipumps, regardless of whether HRP or vehicle was infused (data not shown). Mean arterial pressure, measured by indwelling catheters in conscious rats 14 days later, was highly increased in 2K1C animals compared with sham-operated rats (Figure 1). There was no effect of HRP on mean arterial pressure. However, marked left ventricular hypertrophy was present in all 2K1C rats, regardless of treatment (Figure 1).

Renin mRNA was increased in clipped kidneys and decreased in contralateral kidneys of 2K1C rats (Figure 2A). There was no effect of the peptide on renin mRNA levels. The clipped and contralateral kidney of vehicle or HRP-treated 2K1C rats showed the same (P)RR mRNA expression, which was not different from sham control kidneys (Figure 2B). Kidney renin protein, as measured by the juxtaglomerular index, was regulated in parallel to renin mRNA. A higher

Table. Body and Kidney Weights

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Left Kidney, mg/g</th>
<th>Right Kidney, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>5</td>
<td>365±11</td>
<td>3.08±0.15</td>
<td>3.00±0.09</td>
</tr>
<tr>
<td>2K1C vehicle</td>
<td>7</td>
<td>323±14</td>
<td>3.09±0.21</td>
<td>4.57±0.24*</td>
</tr>
<tr>
<td>2K1C + HRP</td>
<td>5</td>
<td>318±24</td>
<td>2.88±0.24</td>
<td>4.53±0.54*</td>
</tr>
</tbody>
</table>

Data are means±SEMs. *Significant (P<0.05) differences vs sham controls. There were no significant differences between 2K1C vehicle and 2K1C+HRP-treated groups.

![Figure 1. Mean arterial pressure (left) measured in conscious rats via indwelling arterial catheters and relative left ventricular weight (right) measured after sacrifice. Data are means±SEMs. *Significant (P<0.05) differences vs sham controls. There were no different significances between 2K1C vehicle and 2K1C+HRP-treated groups.](image)
percentage of renin-positive glomeruli was found in clipped kidneys and a lower percentage in nonclipped, contralateral kidneys, regardless of whether the HRP had been administered (Figure 3). Plasma renin activity was increased in 2K1C rats compared with sham-operated controls (Figure 4). The increase was smaller in HRP-treated 2K1C rats than in vehicle-infused 2K1C rats, and a statistically significant difference with sham-operated controls was present only in vehicle-infused 2K1C rats (Figure 4). However, plasma aldosterone was increased to the same extent in vehicle-infused and HRP-treated 2K1C rats compared with sham controls (Figure 4).

The relative weight of the contralateral, nonclipped kidney was increased in 2K1C animals compared with sham controls and was not affected by treatment (Table). Examination of periodic acid Schiff–stained sections did not show any difference in the extent of hypertensive nephrosclerosis in the contralateral, nonclipped kidneys of vehicle-infused versus HRP-treated 2K1C rats (Figure 5). Severe vascular lesions indicative of malignant hypertension were observed in 2 of 5 HRP-treated and in 3 of 7 vehicle-treated 2K1C rats, respectively. We found no difference in 2 indices of hypertensive nephrosclerosis. Interstitial macrophage infiltration and interstitial collagen I accumulation were markedly altered in all of the 2K1C animals but no difference between the vehicle and the HRP groups was identified (Figure 6).

**Discussion**

The major finding of the present study is that chronic HRP treatment did not improve target organ damage in renovascular Goldblatt hypertensive rats. 2K1C rats develop high renin, prorenin, and PRA leading to Ang II–dependent target organ damage. Renin is elevated for ≥4 weeks after clipping, and target-organ damage develops during the second 2-week period. We thought that these mechanisms would be blocked by competitive binding of HRP to the (P)RR during this time period, resulting in improved target organ damage. However, longer treatment periods could be necessary to regress existing damage. Nonproteolytic activation of prorenin, local cardiac, and renal Ang II generation, as well as direct (pro)renin-(P)RR mitogen-activated kinase activation, should all have been diminished after HRP treatment with the consequence of improved renal and cardiac damage. Our data clearly yielded no evidence for an amelioration of target organ damage. We very recently performed a detailed analysis of (pro)renin-(P)RR signaling in monocytes. Renin and prorenin both induced extracellular signal–regulated kinase 1/2 phosphorylation in an Ang II–independent manner. However, neither renin- nor prorenin-induced extracellular signal regulated kinase 1/2 signaling could be blocked by HRP. We also demonstrated that embryonic stem cells with a gene trap for the (P)RR, which lacks the transmembrane domain, nonetheless bound HRP. This finding indicates that HRP binding was not related to the (P)RR. We next extended our analysis and investigated the role of HRP on (pro)renin binding. 125I-renin and 125I-prorenin binding in monocytes were also independent of HRP. Our in vivo and in vitro data question the use of HRP as a (P)RR blocker, although Ichihara et al provided a series of studies, predominantly in experimental type 1 diabetes, where HRP treatment improved nephropathy.

We can only speculate about the discrepancy of these results. However, it is obvious that diabetic complications correlate with high prorenin levels and not PRA, pointing to an exclusive role for prorenin. In diabetic patients and in diabetic rats and mice, high prorenin and low renin conditions are the rule. In contrast, renovascular hypertensive rats are characterized by high renin and high prorenin levels. Renin and prorenin both bind to the (P)RR and promote actions of the receptor. Because HRP consists of a 10 amino acid sequence of the prosegment of prorenin, the question arises regarding how this sequence could act as a competitive receptor blocker for renin, which lacks the prosegment. However, whether a high prorenin:renin ratio could deter-
mine the actions of HRP is speculative. Thus, we cannot exclude the possibility that HRP is a blocker for prorenin in situations, where prorenin is elevated but renin and Ang are suppressed. Nevertheless, the data of Ichihara et al.\(^{11}\) in spontaneously hypertensive rats argue against this hypothesis. They showed that HRP treatment reduced local cardiac Ang II levels leading to reduced cardiac hypertrophy and fibrosis.\(^{11}\) Current notions suggest that cardiac Ang II depends to a large extent on renal renin that is released in its active form and is taken up by the heart and, thus, initiates local Ang II generation.\(^{22,23}\) Susic et al.\(^{24}\) also treated spontaneously hypertensive rats with HRP. They described an amelioration of left ventricular hypertrophy; however, they did not confirm such an effect on cardiac collagen content, left ventricular function, and coronary and renal hemodynamics. Altogether, we believe that HRP efficacy in vivo depends on an undefined mechanism but not on competitive antagonism for the (P)RR.

Recently, Schefe et al.\(^{25}\) reported that renin-(P)RR activation in cardiomyoblasts results in the nuclear translocation of the transcription factor promyelocytic zinc finger protein leading to (P)RR downregulation. However, this mechanism obviously does not occur in renovascular hypertension. Krebs et al.\(^{18}\) demonstrated very recently that excessive blood pressure lowering in 2K1C rats resulted in a huge upregulation of renin and prorenin, which was accompanied by (P)RR upregulation in the clipped kidney. These changes were apparently accompanied by substantial ischemia-induced injury to the clipped kidney.\(^{26}\) The pathogenesis of ischemic damage, however, differs from that of hypertension-induced renal injury in the clipped kidney exposed to high blood pressure.\(^{26}\) In addition, we found in the present study that neither the clipped nor the contralateral kidney differed in their (P)RR expression from sham controls. Also HRP treatment did not affect renal (P)RR mRNA expression.

**Perspectives**

Whether the (P)RR is a receptor component of the renin-Ang system with a limited role in cardiovascular regulation or whether the protein fulfills additional function(s) fundamental

![Graph](image-url)

**Figure 4.** Plasma renin activity (left) and plasma aldosterone (right) measured by radioimmunoassay. Data are means±SEMs. *Significant (P<0.05) differences vs sham controls. There were no significant differences between 2K1C vehicle and 2K1C+HRP-treated groups.

![Photomicrographs](image-url)

**Figure 5.** Representative photomicrographs of periodic acid Schiff-stained sections from the right (nonclipped) kidney of a sham-operated rat (A), a vehicle-treated 2K1C rat (B), and an HRP-treated 2K1C animal (C). D, Example of severe vascular lesions. This photomicrograph is from a 2K1C+HRP rat, but similar lesions were also observed in vehicle-treated 2K1C (see text). Bar represents 100 μm.
for cell biology needs to be elucidated in the future. Nevertheless, several implications suggest this notion. In silico research showed that the (P)RR is highly homologous in human, mouse, and rat, as well as in chicken, fish, xenopus, and Caenorhabditis elegans. A 8.9-kDa fragment of the (P)RR is also associated with V-ATPase. Indeed, ablation of the (P)RR gene in embryonic stem cells is not compatible with their participation in embryonic development after injection into blastocysts, and its inactivation before the end of embryogenesis is lethal in zebrafish. This state of affairs suggests that the receptor has an important conserved function. Another indication derives from work from Ramser et al; these investigators found that patients with a mutation in the (P)RR gene develop epilepsy with mental retardation. Interestingly, a similar finding has been described for a person with an absent Ang II type 2 receptor. The (P)RR is a novel, intriguing receptor. Specific (P)RR blockers, as well as the generation of (P)RR-deficient mice, will elucidate the function in cardiovascular disease and cell biology.

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