Effects of Human Prorenin in Rats Transgenic for Human Angiotensinogen

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Abstract—The physiological role of prorenin is unknown; however, the possibility that prorenin inhibits renin locally has been suggested. We tested the hypothesis that prorenin may be an endogenous competitor for renin uptake in the tissue. We also investigated whether prorenin can be activated to active renin and affect mean arterial pressure (MAP). Isolated perfused hindquarters of rats transgenic for human angiotensinogen were infused with human renin and/or prorenin. The plateau phase of angiotensin (Ang) I release 15 minutes after cessation of infusions was used as a parameter for renin uptake. Renin (10 ng/mL for 15 minutes) caused sustained release of Ang I (153±19 fmol/mL). Prorenin infusion alone showed no activation to active renin. In addition, we investigated MAP and plasma Ang II levels after injection of saline (ΔMAP, −1±2 mm Hg; 40±5 fmol/mL Ang II), 9 ng renin (ΔMAP, +37±3 mm Hg; 378±39 fmol/mL), and 144 ng prorenin (ΔMAP, +10±5 mm Hg; 61±5 fmol/mL) and the coinjection of renin and prorenin (ΔMAP, +41±4 mm Hg; 305±23 fmol/mL) in anesthetized rats. The data show that prorenin was not activated to active renin and did not affect MAP in short-term experiments. Renin-induced Ang formation was not affected by prorenin. Renin may have been taken up specifically because of its physical and chemical properties or because of nonspecific sequestration in the extravascular space. We conclude that prorenin does not act as an endogenous antagonist for the long-lasting effects of renin in the vascular wall. Moreover, prorenin does not affect acute renin-related effects on blood pressure. (Hypertension. 1999;33[part II]:312-317.)

Key Words: rats, transgenic ■ angiotensinogen ■ angiotensin ■ prorenin ■ renin ■ hindlimbs

Circulating renin, a key regulatory enzyme of the renin-angiotensin system (RAS), plays a major role in cardiovascular homeostasis, including the regulation of blood pressure, salt balance, and tissue remodeling. Angiotensin (Ang) formation has been reported to occur both in the circulation and at tissue sites. In fact, the tissues are the major site of Ang I and Ang II formation, and the release of locally formed Ang II contributes to the circulating levels of these peptides.1–3 Kidney-derived renin is the major source of tissue renin.4 Renin can be taken up by several tissues to react locally with its substrate angiotensinogen (Aogen).5,6 Several renin-binding proteins have been described; nevertheless, the mechanism of renin uptake is still unclear.7–10 Sealey et al9 described a prorenin/renin-binding receptor. By competing for binding to this receptor or to other receptors, prorenin may be a natural antagonist of tissue-directed RAS.

Prorenin is the biosynthetic precursor of renin. In humans, as much as 90% of circulating renin exists in its enzymatically inactive form.11 However, prorenin is both the primary and final product of renin gene expression in most tissues.12–14 Juxtaglomerular cells of rat and human kidneys constitute the main site of prorenin synthesis and the exclusive site of its intracellular processing to active renin. After bilateral nephrectomy, plasma prorenin levels fall >50% within several hours and remain at this level indefinitely, indicating that other tissues are also involved in prorenin synthesis.15,16 In vitro, prorenin can be activated to active renin by cryoactivation, acid activation, or proteolytic activation.11,15,17–21 Recently, van Kesteren et al10 reported that prorenin is taken up in neonatal rat cardiac myocytes and fibroblasts by the mannose-6-phosphate receptor followed by intracellular activation. However, the role of prorenin in physiology and pathology is still unknown.

The goal of our study was to evaluate the local and systemic effects of human renin and/or human prorenin. We investigated the uptake and local activation of prorenin in the vascular wall of the isolated perfused hindlimb model. We also tested the hypothesis that prorenin may act as an endogenous competitor for renin-related effects in the tissue. We then investigated whether prorenin can be activated in vivo and affect mean arterial pressure (MAP). To avoid confounding effects of endogenous renin produced by the rat,
we used rats harboring the human Aogen gene as a pharmacological model. Pharmacological concentrations of human renin and/or prorenin were infused to facilitate the detection of Ang formation from Aogen. In our study, we focused only on acute effects. Prorenin was not activated to active renin and did not affect MAP. Furthermore, renin uptake and vascular Ang formation were also not affected by prorenin.

Methods

Animals
Male and female heterozygous Sprague-Dawley rats harboring the complete human genomic Aogen gene, TGR(hAOGEN) 1623, and weighing 300 to 400 g were used for all experiments. The transgenic line was developed as outlined elsewhere.22 The rats were kept in rooms at 24±2°C and were fed a standard rat diet (No. C-1000, Altromin) containing 0.2% sodium by weight and were allowed free access to tap water. All procedures were done according to guidelines from the American Physiological Society and were approved by local authorities (AZ IV A/45-G 0399/92 and AZ IV A/45-G 02294).

Hindquarter Perfusion
Preparation and perfusion were performed with rats under thiopental anesthesia (75 mg/kg IP) as previously described.6 Briefly, rats underwent median laparotomy. After evisceration, the abdominal aorta and the inferior vena cava were cannulated, and the perfusion was started immediately. Two hindquarters were perfused in parallel in a nonrecirculating system with a modified Tyrode’s solution containing 0.2% sodium by weight and were allowed free access to tap water. All procedures were done according to guidelines from the American Physiological Society and were approved by local authorities (AZ IV A/45-G 0399/92 and AZ IV A/45-G 02294).

Experimental Protocols

Hindlimb Perfusion
After an initial 30-minute period of baseline perfusion, perfusate for measurement of peptides was collected after 3, 6, 9, 12, 15, 20, 25, 30, 35, 40, and 45 minutes. All perfusate samples were collected over a period of 9 seconds in the presence of a cocktail containing 26 mmol/L phenanthroline (Sigma Chemie), 125 mmol/L EDTA (Sigma Chemie), and a human specific renin inhibitor (remikiren, Hoffmann-La Roche) (10^{-3} mol/L) to prevent any Ang formation outside the hindlimb. Remikiren completely blocks renin activity during sample collection and handling. Enzyme-kinetic renin measurements were performed without remikiren. Purified human recombinant renin and prorenin produced in a Chinese hamster ovary expression system23 were infused for 15 and 20 minutes, respectively. Human recombinant prorenin always exhibited a small active renin contamination; therefore, we used the term “total renin.” The samples were immediately frozen on dry ice and stored at −80°C until assayed. One sample was obtained after the washout period of each experiment to exclude contamination of the perfusion system with human renin and Ang peptides.

Vascular Ang I Release
This protocol was performed to investigate whether prorenin can be activated to active renin and generate Ang I from Aogen in the hindlimb preparation. Human recombinant prorenin (15 ng/mL) was infused for 15 minutes, and the perfusate was collected over a period of 9 seconds as described above in 6 hindlimb preparations. Renin infusions were stopped after 15 minutes; collections were continued to 45 minutes. The perfusate contained 10^{-3} mol/L captopril to inhibit any conversion of Ang I to Ang II. We also investigated the effect of prorenin on renin-induced vascular Ang I formation. In 4 separate experiments, human renin (10 ng/mL) was infused for 15 minutes. A 20-minute infusion of prorenin was started 2 minutes before renin infusion, and the perfusate was collected as described above.

Effects of Renin and/or Prorenin on MAP and Plasma Ang II Levels
The protocol was performed in anesthetized rats transgenic for human Aogen (pentobarbital 60 mg/kg IP) to determine the effects on MAP and plasma Ang II levels. Either saline, renin (9 ng), or prorenin (9, 20, and 154 ng total renin) was injected in 5 to 12 experiments at each dose into the vena jugularis. MAP was recorded with a pressure transducer connected to an implanted catheter (in the arteria femoralis) and on-line computer system (TSE). In 3 to 5 experiments, 9 and 154 ng total renin were injected, followed by a 9-ng renin injection 25 minutes after the first bolus. Blood (600 mL) was withdrawn via the arteria femoralis 15 minutes after each injection in the presence of a cocktail containing 26 mmol/L phenanthroline (Sigma Chemie) and 125 mmol/L EDTA (Sigma Chemie) and a human specific renin inhibitor (remikiren, Hoffmann–La Roche, 10^{-3} mol/L) to prevent any ex vivo Ang formation. Blood withdrawal did not affect MAP. The volume was replaced by saline. Blood samples were centrifuged, frozen on dry ice, and stored at −80°C until analyzed.

Enzyme-Kinetic Determinations and RIA
Immunoreactive Ang I and Ang II concentrations were determined by direct radioimmunoassay (RIA). Details of the RIA are described elsewhere.6 There was no interference of remikiren or captopril in these immunoassays. The limit of detection was 2 fmol/mL for Ang II and 0.1 ng Ang I · mL^{-1} · h^{-1} for human renin.

Human renin concentration was determined by a similar enzyme-kinetic assay.6 Plasma from 48-hour, bilaterally nephrectomized TGR(hAOGEN) 1623 rats was used as a source of excess renin substrate. All samples were incubated together with nephrectomized transgenic rat plasma at pH 5.7 and 37°C for 1 or 2 hours. Rat Aogen was not cleaved during incubation because of the absence of any detectable rat renin in the perfusate. Ang I generated during the enzyme-kinetic assays was measured by direct RIA, and human renin concentration was expressed as pmol Ang I · mL^{-1} · h^{-1}.

Activation of Inactive Renin (Prorenin) by Trypsin
Perfusion (25 µL) was incubated at 4°C for 10 minutes with 25 µL trypsin (40 µg/mL) in 0.1 mol/L sodium phosphate, pH 7.5, containing 0.1% BSA. The reaction was stopped by the addition of 100 µL of soybean trypsin inhibitor (1 mg/mL) in 0.2 mol/L sodium phosphate/citrate buffer, pH 5.7, containing 0.1% BSA and 2% EDTA.

Determination of Prorenin
From each sample, renin concentration (total renin) after its activation by trypsin and the plasma renin concentration were determined. Direct renin measurements were performed with an IRMA Pasteur kit (Sanofi Pasteur) according to the manufacturer’s instructions. The amount of human prorenin was calculated by subtracting the amount of human renin from each sample of the total renin amount after activation of inactive renin. The amounts of human prorenin and human renin of the infused or injected stock solutions were determined before the experiments.

Statistical Analysis
Data are expressed as mean±SEM. Repeated-measures ANOVA was used to study the influence of renin and/or prorenin infusion and time of perfusion on Ang I release and changes in MAP, followed by unpaired Student’s t test as a post hoc test. Statistical significance between protocols with coinfusion of inhibitors was estimated by 1-way ANOVA and Scheffe’s test. A numerical value of \( P<0.05 \) was accepted as significant.
Results

The perfusion pressure of the isolated rat hindquarters ranged from 18 to 22 mm Hg. No Ang I or II could be measured in samples taken before the infusion of human renin and/or prorenin. Addition of renin (10 ng/mL) to the perfusate led to release of readily detectable amounts of Ang I from the perfused rat hindquarters (Figure 1). These experiments were performed in the presence of captopril; no change of perfusion pressure was detected. Renin caused no Ang I formation if infused into nontransgenic rat hindquarters or into perfusion channels without hindquarter preparation.

The time course of renin and Ang I concentration after cessation of renin infusion (Figure 1) provided evidence for uptake of renin to vascular tissue. Renin in venous perfusate decreased sharply to barely detectable levels within 5 to 10 minutes, whereas Ang I release remained elevated and constant (Figure 1). The enzyme was clearly still active at the vascular wall despite its absence from the perfusate. The infusion of 15 ng/mL of total renin (14 ng/mL prorenin; 1 ng/mL renin) caused no detectable Ang I release from the preparation. No activation of prorenin to active renin was seen in these experiments. We also tested whether human prorenin may act as an endogenous competitor for renin uptake in the tissue (Figure 2). Coinfusion of human renin with a 15-fold excess of total renin (144 ng/mL prorenin) did not affect local vascular Ang I release (117±10 versus 123±12 fmol/mL 30 minutes after cessation of the infusion).

We then investigated the effects of human prorenin and/or human renin on MAP. Saline bolus injection in anesthetized male rats transgenic for human Aogen did not affect MAP (ΔMAP, −1±2 mm Hg). Total renin injections of 9, 20, and 154 ng did not increase MAP (1±2, 4±3, and 10±5 mm Hg, respectively). Conversely, 9 ng of active renin caused a significant increase of 37±3 mm Hg (Figure 3A). To investigate whether prorenin could influence the renin-induced pressure response, we first injected either 9 or 154 ng total renin, which was followed by a 9-ng renin bolus 15 minutes later. Both protocols showed a similar increase in MAP (Figure 3A), which was not different from renin alone (46±4, 41±4, and 37±3 mm Hg, respectively). Human renin and/or prorenin injections in female TGR(hAOGEN)1623 rats show a similar picture (data not shown). Plasma Ang II levels were shown in Figure 3B. Total renin injections (9, 20, and 154 ng) showed no differences in plasma Ang II compared with saline (55±2, 40±21, and 61±5 fmol/mL versus 40±5 fmol/mL, respectively). Ang II levels after renin injection alone were significantly increased compared with saline or total renin injections but were not different from plasma Ang II levels with total renin plus renin injections (Figure 3B). The effects of time course on MAP after subsequent bolus injections of either saline plus 9 ng active renin or 154 ng total renin plus 9 ng active renin are shown in Figure 4. Both protocols showed a similar pattern, indicating that prorenin was not activated and that it did not affect renin-induced effects on MAP.

Discussion

The physiological role of prorenin is unknown; however, the possibility that prorenin inhibits the effects of renin locally has been raised.8 We tested the hypothesis that prorenin may be an endogenous competitor for renin uptake in the tissue. We also investigated whether prorenin can be activated to active renin and affect MAP. Therefore, we used transgenic rats with a high overexpression of human Aogen22 to study the formation of Ang peptides at the level of the vascular wall and in the circulation. We infused human recombinant renin and prorenin generated by Chinese hamster ovary cells,23 which show similar properties with respect to molecular weight, kinetic activity, activation and immunological characteristics, and binding to a Cibacron blue affinity column compared with human plasma and renal inactive renin.24 Our study focused on acute effects. Prorenin was not activated to active renin at the site of the tissues or in the circulation. MAP was neither increased nor decreased after prorenin injections. Furthermore, renin-induced Ang formation was also not affected by prorenin. We cannot state whether renin was taken up specifically because of its physicochemical properties or because of nonspecific sequestration in the extravascular space. We conclude that prorenin does not act as an endogenous antagonist for the long-lasting effects of renin in the vascular wall.
Evidence for renin binding to several receptors or proteins has been reported by several investigators, who suggested that renin is actively taken up by tissues or into the cell. The extent of binding as well as the consequences of the binding differ markedly between organs. Sealey et al. described a prorenin-binding protein, with the highest binding capacity of membranes in the kidney, liver, and testis but low binding capacity in heart and aorta and almost none in skeletal muscle. Therefore, the possibility that the prorenin-binding protein is a candidate for interaction in our model is not very likely. Nevertheless, the injection of prorenin and/or renin into intact animals in our in vivo experiments addressed the possible interaction with the prorenin-binding protein. However, we found no evidence for an increase or decrease in MAP after prorenin injection in intact rats. This finding is in line with the unaltered plasma Ang II levels. No changes in MAP or plasma Ang II were observed after subsequent injections of prorenin and renin. This observation speaks against the notion that prorenin may regulate MAP and/or plasma Ang II levels in our short-term experiments.

Campbell and Valentijn reported on a different renin-binding protein in vascular tissue. They described the highest binding capacity in mesenteric arteries. Thus, we speculated that renin and/or prorenin might bind to this protein in our isolated perfused resistance vessel bed. Our present data clearly demonstrate an uptake of human renin into vascular tissue and a local action, because the effects of renin were still present after cessation of renin infusion, when renin activity in the perfusate was almost undetectable. The infusion of prorenin did not cause any vascular Ang formation. However, vascular Ang formation was not different after competition of prorenin with renin for a putative receptor, compared with renin-induced Ang formation. Therefore, we are tempted to speculate that renin and prorenin do not bind to the same receptor. Alternatively, renin uptake could be mediated by nonspecific sequestration in the extravascular space.
ever, we believe that the long-lasting renin-induced local Ang formation is of (patho)physiological importance, irrespective of the mechanism of uptake. We cannot completely exclude the possibility that human renin binding and/or human prorenin binding to rat binding proteins could be hampered by species specificity. However, we and others have obtained similar results for local Ang formation with different renin sources in the rat model.

Recently, Hu et al.30 showed identical hemodynamic and hormonal responses to 14-day infusions of renin or Ang II in conscious rats. They concluded that tissue-bound renin does not play a unique role in promoting cardiovascular, renal, hormonal, or drinking responses. However, several investigators, including ourselves,31,32 have shown that Ang II–induced end-organ damage in the kidney and heart is not only influenced by systemic blood pressure and circulating Ang II levels.

Activation of prorenin was reported by several groups. Hsueh et al.34 identified a 25-kDa enzyme in the kidney, which was able to activate human recombinant prorenin. Trypsin, plasmin, pepsin, kallikrein, and other proteases can also activate prorenin.11,33,34 However, some of these enzymes are unlikely candidates to process active renin, because, for example, trypsin and pepsin also degrade renin. Recently, van Kesteren et al.10 reported that human renin and human prorenin were taken up into neonatal rat cardiac myocytes and fibroblasts by the mannose-6-phosphate receptor. Prorenin was activated in the cell within a few minutes. Nevertheless, it remains unclear whether intracellularly activated prorenin only contributes to intracellular communication or is secreted back into the circulation or whether this pathway is merely an extracellular mechanism of degradation. Experiments in primates showed that the liver and kidney may take up circulating prorenin and convert prorenin to active renin without secreting renin back into the circulation.34 The unchanged release of Ang in the perfusate and unaltered plasma Ang II levels we observed could be explained by such mechanisms.

Experimental and epidemiological data have demonstrated an important role for the RAS in the pathogenesis of hypertension and end-organ damage. However, the role of prorenin in the pathogenesis of hypertension and end-organ damage remains uncertain.12,35 although elevated plasma prorenin concentrations have been implicated as a cause of microangiopathy in diabetic patients.36 A newly developed transgenic rat model developed by Veniant et al.37 that overexpresses rat prorenin in the liver exhibited a 400-fold increase in plasma prorenin. The transgenic model showed cardiac hypertrophy and severe renal lesions in the absence of blood pressure. Thus, the model demonstrated that long-term exposure to elevated plasma prorenin is vasculotoxic. One possible explanation is that during the inflammatory process, neutrophils produce abundant enzymes that possibly activate prorenin and thereby enhance local Ang II levels.38 Our results are limited by the fact that we focused on only short-term effects. Nevertheless, the very rapid activation of prorenin in vitro would predict detectable short-term effects.

In summary, our data showed that human recombinant prorenin was not activated to active renin at the site of the vascular tissue or in the circulation. In the isolated perfused hindquarter, human renin can be taken up from the circulation and remains active much longer than its presence in the circulation would explain. The renin-induced vascular Ang I formation was not influenced by prorenin, indicating that prorenin is not acting as an endogenous competitor for renin uptake to the vascular wall. In vivo, MAP was neither increased nor decreased after prorenin injections. Competition of prorenin and renin did not affect MAP and plasma Ang II compared with renin injection alone. Therefore, we conclude that prorenin does not act as an endogenous antagonist of renin for its uptake in the vascular wall and does not influence renin-induced effects on blood pressure in short-term experiments. Further studies are necessary to determine the importance of intracellular activation of prorenin and its long-term effects on end-organ damage.

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