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Receptor-mediated nonproteolytic activation of prorenin and induction of TGF-β1 and PAI-1 expression in renal mesangial cells

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Zhang J, Wu J, Gu C, Noble NA, Border WA, Huang Y. Receptor-mediated nonproteolytic activation of prorenin and induction of TGF-β1 and PAI-1 expression in renal mesangial cells. Am J Physiol Renal Physiol 303: F11–F20, 2012. First published April 25, 2012; doi:10.1152/ajprenal.00050.2012.—While elevated plasma prorenin levels are commonly found in diabetic patients and correlate with diabetic nephropathy, the pathological role of prorenin, if any, remains unclear. Prorenin binding to the (pro)renin receptor [(p)RR] unmask prorenin catalytic activity. We asked whether elevated prorenin could be activated at the site of renal mesangial cells (MCs) through receptor binding without being proteolytically converted to renin. Recombinant inactive rat prorenin and a mutant prorenin that is noncleavable, i.e., cannot be activated proteolytically, are produced in 293 cells. After MCs were incubated with 10^{-7} M native or mutant prorenin for 6 h, cultured supernatant acquired the ability to generate angiotensin I (ANG I) from angiotensinogen, indicating both prorenin was activated. Small interfering RNA (siRNA) against the (p)RR blocked their activation. Furthermore, either native or mutant rat prorenin at 10^{-7} M alone similarly and significantly induced transforming growth factor-β1, plasminogen activator inhibitor-1 (PAI-1), and fibronectin mRNA expression, and these effects were blocked by (p)RR siRNA, but not by the ANG II receptor antagonist, saralasin. When angiotensinogen was also added to cultured MCs with inactive native or mutant prorenin, PAI-1 and fibronectin were further increased significantly compared with prorenin or mutant prorenin alone. This effect was blocked partially by treatment with (p)RR siRNA or saralasin. We conclude that prorenin binds the (p)RR on renal MCs and is activated nonproteolytically. This activation leads to increased expression of PAI-1 and transforming growth factor-β1 via ANG II-independent and ANG II-dependent mechanisms. These data provide a mechanism by which elevated prorenin levels in diabetes may play a role in the development of diabetic nephropathy.

Prorenin; fibrosis; mesangial cell; diabetic nephropathy

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Diabetic nephropathy (DN), a major long-term microvascular complication of types 1 and 2 diabetes, is the leading cause of end-stage renal disease in the United States and contributes to significant morbidity and mortality of diabetic patients. Numerous factors contribute to the pathogenesis and progression of DN (23). In studies that range from therapeutics to pharmacology to genetics, many lines of evidence have strongly suggested that activation of renin-angiotensin system (RAS) in diabetes contributes to DN; hence pharmacological blockade of the RAS has become a standard and essential therapy in the management of DN (26). Prorenin is a precursor of renin. Elevated plasma prorenin levels are commonly found in diabetic patients, and it has been known for more than 20 years that prorenin levels correlate with microvascular diseases of diabetes, including nephropathy (7, 10, 27, 28). Neither the cause for elevated prorenin levels nor their role in the microvascular disease, if any, is known. Recently, a newly cloned (pro)renin receptor [(p)RR] was identified on human mesangial cells (MCs) and has now been shown to be present in vivo in normal glomeruli and tubules (31, 32). This receptor binds prorenin as effectively as it binds renin, and, when bound, prorenin cleaves angiotensinogen (AGT) with kinetics similar to that of fully active renin in solution without proteolytic removal of its prosegment (32). The discovery of this new (p)RR has sparked interest in whether prorenin bound to this receptor per se, without being proteolytically converted to renin, plays a role in DN.

That (p)RR binding would play a pathological role in diabetes was first hypothesized by the impressive observations of Ichihara’s group (18–21). This group has shown that a decoy peptide corresponding to the handle region of prorenin, named handle region peptide (HRP), which competitively inhibits prorenin binding to its receptor, attenuated the development and progression of hypertension-induced cardiac fibrosis (19, 20) and also completely inhibited the development of DN in different rat models, including angiotensin II (ANG II) type 1 receptor knockout mice (18, 21). However, the decoy peptide HRP is thought to compete with prorenin for receptor binding at a site that is not found on mature renin. The HRP model is unable to explain the findings that the receptor responds to both renin and prorenin, and renin induces synthesis of a number of molecules involved in renal fibrosis via the same receptor (16, 17). Of concern is that several leaders in this field have attempted to reproduce the impressive animal data of Ichihara’s laboratory without success (2, 8, 9, 30). The HRP story, therefore, remains unconfirmed, and the role of (p)RR binding in diabetic compliments remains obscure.

Importantly, the (p)RR not only represents a novel component of the RAS, but is also able to induce a signal-transduction cascade upon ligand binding independent of ANG II generation and action. Binding of renin or prorenin causes activation of mitogen-activated protein kinases, leads to cell proliferation and to upregulation of profibrotic genes expression, and may play an important role in end organ damage (11, 32, 37, 40, 43, 44). Very recently, it has been observed that (p)RR expression is increased in the kidneys of diabetic animals and in MCs stimulated with high concentrations of glucose (13). A key...
question is whether glucose-induced increases in receptor expression contribute to glomerular prorenin activation, receptor-mediated intracellular signaling, and increased production of profibrotic proteins in DN. As a first step, we asked whether prorenin can be directly activated by renal MCs and lead to synthesis of a number of molecules involved in tissue fibrosis, whether (p)RR binding is involved, and whether proteolytic cleavage to renin is required. We utilized native prorenin and mutant prorenin that was noncleavable, i.e., cannot be converted to renin by any known prorenin-renin convertases (e.g., trypsin, cathepsin B, etc.).

**METHODS**

**Reagents**

Rat AGT or normal rat serum was prepared as previously described (44). Other reagents were purchased from Sigma (St. Louise, MO), unless otherwise indicated.

**Expression and Purification of Recombinant Rat Native and Mutant Prorenins**

Recombinant rat native prorenin was produced and purified as described previously (44). The noncleavable mutant rat prorenin gene was made at AA130/131GC and AA133/134/GC, by using the PCR-based site-directed mutagenesis and verified by DNA sequencing. The resulting Ala-substituted mutant rat prorenin was produced and purified by the same method as native rat prorenin (44).

To verify that the mutant rat prorenin produced is incapable of being converted to renin by trypsin, both native rat prorenin and mutant prorenin were applied to agarose-immobilized TPCK trypsin (Pierce, Rockford, IL) for digestion for 6 h at 37°C, according to the manufacturer’s instructions. Trypsin was removed by centrifugation after digestion. Samples were collected before and after trypsin digestion for SDS-PAGE analysis and the measurement of catalytic activity, as described previously (17). Briefly, the recombinant proteins were concentrated and added in loading buffer. Proteins were separated by 10% Tris-glycine gel electrophoresis (Invitrogen Life Technologies, Gaithersburg, MD). Gels were visualized by Coomassie blue dye and photographed. The catalytic activity of prorenin was determined by an ANG I generation assay (44). ANG I was quantitated using a commercially available RIA kit (Phoenix Pharmaceuticals). Cellular RNA was used for (p)RR, transforming growth factor (TGF)-β1, plasminogen activator inhibitor-1 (PAI-1) and fibronectin (FN) mRNA determination by real time RT-PCR. PAI-1 protein levels in the supernatant were determined by Western blot after extension incubation for 36 h, as described (17, 44).

The (p)RR dependence was determined when the (p)RR was depleted by Stealth RNA interference, as described previously (16, 17, 44). Depleted and control MCs were then treated with prorenin or mutant prorenin in the presence or absence of AGT, with or without saralasin. Prorenin activation, cellular mRNA and supernatant ANG I, and PAI-1 were determined again, as described above.

**RNA Preparation and Real-time RT-PCR**

Total RNA was extracted immediately from MCs using Trizol reagent (GIBCO BRL), according to the manufacturer’s instructions. Total RNA (2 μg) was reverse transcribed using the SuperScript III first-strand synthesis system for RT-PCR kit (Invitrogen). Real-time RT-PCR was then performed using SYBR Green I gene assay kit (Applied Biosystems, Foster City, CA) with ABI 7900HT system, as described previously (44). Samples were run as triplicates in separate
wells to permit quantification of the target gene normalized to GAPDH used for equal loading. Sequences of primers used are listed in Table 1. The specificity of PCR products was confirmed on a 1.5% agarose gel by showing a specific single band with the expected size.

**PAI-1 Western Blot Assay**

Forty microliters of cell supernatant were centrifuged and denatured before loading to SDS-PAGE gel. The blots were processed and quantitated, as described previously (14, 44).

**Statistical Analysis**

Values are expressed as means ± SD. Groups were analyzed by one-way ANOVA, and the Student-Newman-Keuls comparison was employed to compare differences among groups. A value of $P < 0.05$ was considered statistically significant. Duplicate wells were analyzed for each experiment, and data were generated from a pool of at least three independent experiments.

**RESULTS**

**Production of Rat Mutant Prorenin**

As shown in Fig. 1A, the purified rat mutant prorenin was clearly present as one clear band with the predicted size of ~43 kDa. Unlike native rat prorenin (17), the mutant rat prorenin could not be digested by trypsin after incubation with TPCK-trypsin for 6 h, as visualized by SDS-PAGE analysis. In contrast, prorenin was cleaved to renin when it was incubated with trypsin (17). The catalytic activity of renin increased more than 1,000-fold after trypsin digestion (Fig. 1B). However, the mutant prorenin had no catalytic activity before or after trypsin digestion compared with rat prorenin. There was no measurable catalytic activity in the control buffer. Together, these data confirmed that the recombinant mutant prorenin, with deficient trypsin binding, could not be cleaved to renin proteolytically. The recombinant rat prorenin and mutant prorenin produced and stored in PBS (pH = 7.4) had no catalytic activity.

**(p)RR Expression in MCs and Normal Glomeruli**

Figure 2 shows that (p)RR mRNA was abundant not only in isolated glomeruli, but also in rat MCs (Fig. 2A). The (p)RR protein was also present in the cell membrane of rat MCs and glomeruli detected by Western blot assay (Fig. 2B). Immunofluorescent staining with a specific anti-(p)RR antibody revealed that (p)RR was detected in glomeruli, renal distal tubules, collecting ducts, and blood vessels (Fig. 2C). These results are similar to those seen in both human and animal kidney tissue (1, 12, 32).

**Catalytic Activation of Native/Mutant Rat Prorenin by Incubation with MCs in Vitro**

When $10^{-7}$ M rat prorenin was incubated with rat MCs for 6 h, rat prorenin gained catalytic activity, and this activity was preserved in the presence of $10^{-5}$ M saralasin, a nonselective ANG II receptor blocker (Fig. 3A). Of note, $10^{-7}$ M rat mutant prorenin that was noncleavable also achieved comparable levels of catalytic activities when it was incubated with rat MCs, and this activity was not affected by the presence of saralasin. Without cells prorenin, mutant prorenin or medium alone produced culture supernatant that was unable to generate any catalytic activity (data not shown). These data suggest that native prorenin and mutant prorenin are activated similarly through binding to MCs.

To determine whether the activation of prorenin or mutant prorenin by MCs was mediated by the (p)RR that was shown to be abundant on MCs in Fig. 2B, we inhibited receptor expression with small interfering RNA (siRNA). Cells were pretreated with Stealth (p)RR siRNA to silence receptor mRNA expression and then treated with $10^{-7}$ M prorenin for 6 h. As previously shown in MCs and vascular smooth muscle cells (VSMCs) (17, 44), transfection of a Stealth siRNA molecule that targeted the (p)RR induced over 70% reduction of receptor mRNA expression at a dose of 250 pmol for 48 h (Fig. 3A). Cell transfection with lipofectamine and 250 pmol Stealth siRNA for 48 h significantly reduced increases in catalytic activity (Fig. 3B). Treatment of cells with Lipofectamine 2000 alone or Opti-MEM medium alone had no effect on the (p)RR mRNA expression and native or mutant prorenin activation. In addition, we have tested that treatment of cells with lipofectamine plus scramble control siRNA had no effect on the (p)RR mRNA expression and native or mutant prorenin activation. In addition, we have tested that treatment of cells with lipofectamine plus scramble control siRNA had no effect on the targeted gene expression (45). These results indicate that both native and mutant prorenin activation is (p)RR mediated.

Our laboratory has shown previously that AGT mRNA was undetectable in cultured rat MCs, and that a reservoir of ANG I was not available to convert to ANG II in cultured MCs treated or untreated with recombinant renin (17). An exogenous rat AGT resource needs to be given to further observe whether activated prorenin or mutant prorenin facilitates angiotensin generation.
Indeed, no detectable ANG I was found in the culture supernatant when the exogenous AGT was absent, although prorenin was activated as shown in Fig. 3. However, when MCs were incubated with 10^{-7} M prorenin and serum as a source of AGT, the culture supernatant was able to generate ANG I, and this action was not affected by the presence of saralasin (Fig. 4, A and B). The mutant prorenin had a similar action of prorenin on ANG I generation when exogenous AGT was added (Fig. 4, A and B). However, no catalytic cleavage of AGT to ANG I was seen without cells, whether 3.75% rat AGT serum or 10^{-7} M native or mutant prorenin were present or not. No ANG I was measurable during the incubation of native or mutant prorenin with AGT without MCs (data not shown). These results indicate that there is no detectable active renin/prorenin or ANG I in the prepared rat AGT serum. The amount of soluble prorenin supposed to be activated under physiological condition (37°C, pH = 7.4) is not enough to induce angiotensin generation in this system. The mutant prorenin binds to MCs and is activated the same as native prorenin. Activated mutant prorenin is still able to facilitate ANG I generation as prorenin when exogenous AGT is present. siRNA against the (p)RR blocked the activation of native and mutant prorenins and subsequent facilitation of ANG I generation (Fig. 4, C and D). These results indicate that prorenin activation and ANG I generation are (p)RR mediated. The mutant prorenin acts similarly to native prorenin.

Together, these findings suggest that prorenin is activated through binding to MCs. This activation is not due to proteolytic cleavage of prorenin. It is due to a nonproteolytic mechanism and mediated by the (p)RR.
Effect of Activated Native and Mutant Prorenins on mRNA Expression of TGF-β1, PAI-1, and FN by MCs

Independent of ANG II. Figure 5 shows that both 10^{-7} M prorenin and 10^{-7} M mutant prorenin treatment for 6 h stimulated MC overexpression of TGF-β1, PAI-1, and FN mRNA similarly. With 10^{-7} M prorenin, TGF-β1, PAI-1, and FN mRNA levels were increased 223, 496, and 399%, respectively, by MCs after 6-h incubation (P < 0.05) (Fig. 5, A–C). With 10^{-7} M mutant prorenin, TGF-β1, PAI-1, and FN mRNA levels were increased 207, 430, and 387%, respectively, by MCs after 6-h incubation (P < 0.05). As seen in Fig. 5, A–C, treatment of cells with the nonselective ANG II receptor blocker, saralasin, had no effect on TGF-β1, PAI-1, and FN mRNA levels, either in the absence or presence of native or mutant prorenin, indicating that blocking the action of any ANG II present does not alter prorenin’s effect. In contrast, targeting rat (p)RR with siRNA significantly suppressed the (p)RR mRNA expression (Fig. 6A) and subsequently blocked both native and mutant prorenin-induced increases in TGF-β1, PAI-1, and FN mRNA expression (Fig. 6, B–D). These results indicate that 10^{-7} M native or mutant prorenin induces TGF-β1, PAI-1, and FN mRNA expression by MC through a receptor-mediated, ANG II-independent mechanism.

ANG II-dependent effects. As observed above (Fig. 4), AGT is required for activated prorenin to generate angiotensin. It also has been shown previously that prorenin dramatically stimulates PAI-1 mRNA expression in a manner dependent on the AGT concentration when the effect of exogenous AGT on PAI-1 expression was investigated (44). AGT 3.75% has been chosen experimentally for evaluation of the ANG II-dependent effect of activated prorenin in culture (44). As shown in Fig. 7, B–C, adding both 3.75% AGT and 10^{-7} M prorenin dramatically increased PAI-1 mRNA expression by 38.6-fold, FN mRNA by 3.9-fold, compared with none added serum-free medium, which were much greater than either 10^{-8} M ANG II alone (22) or 10^{-7} M prorenin alone, indicating an additive or synergistic effect of both prorenin and ANG II on PAI-1 expression in MCs. Similarly, mutant prorenin and AGT added together produced marked increases in PAI-1 (33.1-fold) and FN (6.9-fold) mRNA expression compared with serum-free medium. The increases seen were much greater than those seen with 10^{-7} M mutant prorenin alone, suggesting an additive or synergistic effect of mutant prorenin and ANG II on PAI-1 and FN expression in MCs as well. However, 3.75% AGT serum alone had no effect on PAI-1 and FN mRNA expression compared with 3.75% normal rat serum (data not shown).

Effect of Activated Native and Mutant Prorenins on mRNA Expression of TGF-β1, PAI-1, and FN by MCs

Fig. 4. Effects of Sara and (p)RR on activation of native or mProR and subsequent angiotensin generation in cultured MCs when AGT is present. 10^{-7} M Sara had no effect on catalytic activity (A) and ANG I generation (B) in the culture supernatant when cellular (p)RR was present and treated with 10^{-7} M inactive ProR or mProR in the presence of 3.75% rat AGT serum for 6 h, detected by RIA. *P < 0.05 vs. no ProR or mProR additive but AGT-treated Con. Catalytic activity (C) and ANG I (D) generation in the culture supernatant when cellular (p)RR was deleted by Stealth siRNA, followed by treatment of 10^{-7} M inactive ProR or mProR in the presence of 3.75% rat AGT serum for 6 h, detected by RIA. *P < 0.05 vs. the Lip-transfected, no ProR or mProR additive, but AGT-treated Con. Values are means ± SD.

Fig. 5. Effect of Sara (Sar) on ProR or mProR-induced mRNA expression of transforming growth factor (TGF)-β1 (A), plasminogen activator inhibitor-1 (PAI-1; B), and fibronectin (FN; C) by MCs. 10^{-7} M Sar had no effect on 10^{-7} M ProR or mProR induced TGF-β1 (A), PAI-1 (B), and FN (C) mRNA expression determined by real-time RT-PCR. mRNA values are expressed relative to the no-additive Con, which was set at unity. Values are means ± SD. *P < 0.05 vs. Con.
shown), which was consistent with the results that no detectable active renin/prorenin or ANG I was present in AGT serum (Fig. 4).

When $10^{-7}$ M native or mutant prorenin and 3.75% AGT were coincubated, TGF-β1 mRNA expression by MCs was further increased by 130 and 120.6% compared with native or mutant prorenin alone (Fig. 7A). However, the increases in TGF-β1 mRNA expression were far less than the increases in PAI-1 and FN mRNA expression when both native or mutant prorenin and AGT were added.

As shown in Fig. 4, A and B, saralasin did not affect prorenin or mutant prorenin-induced TGF-β1, PAI-1, and FN mRNA expression in MCs when AGT was absent. In contrast, either prorenin or mutant prorenin-induced increases in PAI-1 and FN were significantly reduced by saralasin when AGT was present (Fig. 7, B and C). The increased TGF-β1 mRNA expression induced by prorenin or mutant prorenin and ANG II were also decreased by saralasin. When siRNA was used to silence (p)RR (Fig. 8), prorenin or mutant prorenin-induced TGF-β1, PAI-1, and FN mRNA expression was blocked in the presence of AGT (Fig. 8, A–C), indicating that both prorenin and mutant prorenin also induce ANG II-dependent TGF-β1, PAI-1, and FN expression when a source of AGT is available.

Both prorenin- and mutant prorenin-induced, ANG II-dependent and ANG II-independent effects on TGF-β1, PAI-1, and FN mRNA expression are mediated by the (p)RR. However, the proteolytic activation of prorenin is unnecessary for the activation of the (p)RR-mediated cellular signaling.

### Effect of Activated Native and Mutant Prorenins on PAI-1 Protein Production by MCs

In agreement with mRNA expression, prorenin or mutant prorenin alone increased PAI-1 protein production by 172.2 and 171.3%, respectively, compared with serum-free medium control (Fig. 9A). The exaggerated PAI-1 protein production was reduced by 64.3 and 64.2%, respectively, after the (p)RR was depleted with siRNA (Fig. 9A). Moreover, adding 3.75% AGT serum and $10^{-7}$ M prorenin or mutant prorenin together further increased PAI-1 protein production by 189.2 and 180.5%, respectively, compared with prorenin or mutant prorenin alone in the presence of 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown). Pretreatment with $10^{-5}$ M saralasin significantly reversed the induction of PAI-1 protein expression compared with 3.75% normal rat serum (data not shown).
protein production seen in the cotreatment with prorenin or mutant prorenin and AGT to the levels of PAI-1 production seen in prorenin or mutant prorenin alone, treated in the presence of normal rat serum (Fig. 9B). When MCs were pretransfected with specific siRNA targeting (p)RR and the (p)RR was inhibited, the elevated PAI-1 protein expression induced by the coadministration of prorenin or mutant prorenin and AGT was attenuated by 86.9 and 84.4%, respectively ($P < 0.05$ vs. Con). These data together suggest that prorenin itself may nonproteolytically induce PAI-1 protein production in MCs through the (p)RR-mediated, both ANG II-independent and ANG II-dependent, mechanisms.

**DISCUSSION**

The present study reveals that a solution containing $10^{-7}$ M inactive prorenin has no ability to convert AGT to ANG I until it is incubated with MCs. Acquisition of this catalytic ability is mediated through binding to the (p)RR, since suppression of the receptor by RNA interference diminished it. Of note, this study also reveals that a noncleavable prorenin variant that cannot be enzymatically cleaved to renin binds the (p)RR on the MCs and is activated and subsequently facilitates ANG I formation in a manner very similar to prorenin when AGT is present. Together, the observations that the intact prorenin was still present in the cultured supernatant, and no renin conversion was found by end of the experiment, determined by Western blot (44), provide direct evidence for the mechanisms involved in the activation of prorenin. Inactive prorenin binds to (p)RR, is activated nonproteolytically, and then stays in this activated form in the culture supernatant. Under normal physiological conditions, $<2\%$ of prorenin is activated by temperature- and pH-mediated nonproteolytic activation (6). Our laboratory has previously shown, based on angiotensin generation, that the catalytic activity of cell-activated prorenin is $\approx 37.6\%$ of that of renin, much higher than the expected $2\%$ activity of soluble prorenin (44). It appears that prorenin is activated by binding the (p)RR on the surface of renal MCs as it is on VSMCs, in a nonproteolytic manner. Recently, a truncated extracellular NH$_2$-terminal part of (p)RR, called the soluble (p)RR, was found in rat and human plasma and was able to bind prorenin (5). Whether the soluble (p)RR is present in this culture system and contributes to prorenin binding and activation remains to be determined.

Consistent with our in vitro results in MCs are data from in vivo studies in double-transgenic mice overexpressing human native prorenin and human AGT (29). When transgene expres-
It is important to note that MC activation of (p)RR by both native and mutant rat prorenin resulted in similar increases in expression of profibrotic molecules, such as TGF-β1, PAI-1, and FN. These effects were blocked by (p)RR siRNA, but not by the ANG II receptor antagonist, saralasin. These results indicate that, independent of ANG II generation and action, prorenin binds to and activates the (p)RR, inducing intracellular signaling and synthesis of a number of molecules involved in tissue fibrosis. Proteolytic cleavage of prorenin is unnecessary for the (p)RR-mediated signaling.

Results from angiotensin generation also confirm the notion of prorenin-induced, angiotensin-independent effects. The addition of rat AGT did not result in ANG I generation in the medium (Fig. 4). This result suggests that, under these conditions, rat MCs, like cardiomyocytes (41), do not possess enzymes capable of cleaving rat AGT into ANG I. ANG I generation occurred only when rat prorenin or mutant prorenin were added with rat AGT to MCs. Apparently, prorenin’s activation requires binding to (p)RR on MCs, but does not require a prorenin-renin converting enzyme. Addition of rat AGT induced further increases in TGF-β1, PAI-1, and FN mRNA levels and PAI-1 protein. Since saralasin significantly decreased this effect, and Stealth siRNA to (p)RR blocked this effect, production of TGF-β1, PAI-1, and FN was dependent on receptor-mediated prorenin activation and subsequent angiotensin generation and action. These data are consistent with our laboratory’s previous observations in VSMCs (44), and data where ANG II-induced upregulation of PAI-1 expression was seen when human AGT and prorenin were added to neonatal rat cardiomyocytes (40). The presence of both ANG II-dependent and ANG II-independent effects and the magnitude of increases in profibrotic molecules seen when AGT and prorenin/or mutant prorenin were added together suggest the possibility of an interesting synergistic effect between ANG II and prorenin on expression of TGF-β1, PAI-1, and FN. Such interconnections between members of the RAS and profibrotic molecules have been seen previously. For example, ANG II or renin upregulate PAI-1, both directly by upregulating expression of TGF-β and indirectly because TGF-β also increases PAI-1 (17, 22). Increased expression of TGF-β1 and PAI-1 plays an important role in DN (3). It has been suggested that the RAS, TGF-β, and PAI-1 systems act together, providing an “emergency response” to tissue injury (4). When this response is inappropriate, as in diabetes, continued activation of the RAS, TGF-β, and PAI-1 systems leads to progressive tissue injury with fibrosis and ultimately organ failure (4). It is, therefore, quite likely that the elevated prorenin levels seen in diabetes are involved in generating increased TGF-β and PAI-1 levels in glomeruli, which then contribute to diabetes-associated nephropathy.

Further investigations are needed to determine whether receptor-mediated prorenin binding and activation occur in vivo, especially in diabetes. In addition, very recent data suggest that the (p)RR is multifunctional in that (p)RR has additional functions essential for cell survival and proliferation that may be related to the function of the vacuolar H+ -ATPase (25, 34, 36). Several tools are critical to understanding the relationship between elevated prorenin and DN. These tools include generation of conditional prorenin and noncleavable prorenin mutant transgenic animals without the species differences in RAS components in the studies to date and development of selective (p)RR binding antagonists instead of having to rely on animals with total or tissue-specific ablation of (p)RR.

In conclusion, the study presented here further confirms that prorenin binds to the (p)RR on renal MCs, is activated, and may contribute to disease by both angiotensin-dependent and angiotensin-independent mechanisms. Importantly, the proteolytic cleavage of prorenin is unnecessary for its activation and (p)RR-mediated signaling. It is likely that prorenin, in addition to acting as the biosynthetic precursor of renin, acts as an independent fibrotic mediator. Thus the high levels of prorenin...
seen in diabetes may well contribute to the progression of DN. In terms of a therapeutic approach to slow DN, combination of pro(renin) receptor blockade to decrease angiotensin-independent effects with traditional ANG II receptor blockers or inhibitors of renin’s or ACE’s enzymatic activity to decrease angiotensin-dependent actions may hold promise.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


