Review

Physiological Relevance of Renin/Prorenin Binding and Uptake

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There is compelling physiological evidence of binding and uptake of renin and prorenin in tissues. A number of molecules with the ability to bind renin and prorenin have been identified and have been characterized to varying degrees. It remains unclear, however, just how many renin/prorenin binding proteins and receptors exist and what their physiological functions may be. The possible functions of renin/prorenin binding and uptake are manifold, and include clearance of renin and prorenin from the circulation, local generation of angiotensins, activation of prorenin on the cell surface, trafficking of prorenin between cellular and extracellular compartments as part of a complex processing machinery, and signal transduction both *via* direct receptor mediated signaling, and *via* modulation of O-linkage of *N*-acetyl-glucosamine to cellular proteins. Some of these functions may involve single renin/prorenin binding sites or receptors, while others may require multiple binding sites and receptors. This review describes the physiological studies that have provided evidence of renin/prorenin uptake from the circulation, summarizes our knowledge of renin/prorenin binding proteins and receptors, and postulates new roles for renin/prorenin binding and uptake in tissues. (*Hypertens Res* 2005; 28: 97–105)

***Key Words*: renin, prorenin, binding site, receptor, processing**

Introduction

Although the role of plasma renin in controlling blood pressure and fluid homeostasis is well understood, the role of renin in tissues remains speculative. Despite some earlier controversy about the synthesis of renin in tissues, it is now generally agreed that renin found in most tissues is taken up from the circulation and is of renal origin (*1*, *2*). In addition, prorenin, the inactive precursor of renin, is present in plasma at concentrations often higher than those of active renin. Although various roles for prorenin have been proposed (*3*, *4*), a physiological function for prorenin has never been proven. Recent studies suggest that prorenin may be taken up from the plasma and activated either on cell-surface membranes or intracellularly. These proposed mechanisms raise the question of how renin/prorenin enter tissues, whether by passive or active mechanisms, and, if the process is active, whether these mechanisms are specific or non-specific. Although several renin/prorenin binding and uptake mechanisms have been identified (*5–9*), their functions are as yet only poorly defined, and it is not clear whether these sites function alone, together, or in combination with other mechanisms.

The importance of renin uptake into tissues is that it provides a mechanism to generate angiotensin II (Ang II) locally in excess of that produced in the plasma. In addition to its vasoconstrictor effects on vascular smooth muscle cells, Ang II initiates a multiplicity of intracellular signals (*10*) that may cause smooth muscle cell hypertrophy (*11–13*) or hyperplasia (*13–15*), which in turn lead to vascular remodeling (*15*). These effects may also contribute to target organ damage independent of the effects of Ang II on blood pressure (*14*, *16*, *17*).

The goals of this review are to examine the physiological evidence of renin uptake, and to review the binding and

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uptake mechanisms that have been investigated. Although there is an abundance of physiological data suggesting the function of renin/prorenin uptake in tissues, biochemical studies have lagged in characterizing the underlying mechanisms. In this review I have also attempted to synthesize some of the available data to account for known physiological phenomena, and to suggest possible new roles for renin/prorenin binding and uptake in different tissues.

Physiological Evidence of Renin Uptake

The concept of a tissue binding site for circulating renin originates from the seminal studies of Tigerstedt and Bergman, who observed that the pressor effect of renal extracts lasted for hours in bilaterally nephrectomized rabbits, compared to minutes in intact animals (*18*). Later studies confirmed that the prolonged pressor response was due to renin (*19–21*). Further evidence of renin uptake in vascular tissues was provided by Thurston, Swales and coworkers, who demonstrated angiotensin-dependent blood pressure responses in nephrectomized rats despite rapid clearance of renin from the circulation (*22–30*). They postulated that blood pressure was maintained by renin taken up by the vasculature (*22–24*) and showed parallel changes in aortic renin concentration and blood pressure in several rat models: after nephrectomy of Goldblatt two-kidney, one-clip hypertensive rats (*24–26*), following administration of renin to nephrectomized rats (*28*, *29*), and in other hypertensive models (*30*). Similar findings were also reported by Bing and Nielsen (*31*).

Studies of the rate of Ang II production and metabolism in tissues provided further indirect evidence of tissue uptake of renin (*32*, *33*). Campbell and coworkers calculated that the venous levels of Ang II were too high to be accounted for by circulating renin and the clearance rate of Ang II (*32*). Admiraal *et al*. measured the clearance of radiolabeled Ang I across various tissue beds in humans and found that the venous levels of Ang I were higher than expected, consistent with the presence of enzymatically active renin (*33*). One explanation for these findings is that tissue binding of renin could result in the local generation of angiotensin, and thereby in the high levels of Ang II such as those that have been measured in the kidney (*34*, *35*).

Several transgenic studies also support the concept of renin/prorenin uptake from the circulation. First, Veniant *et al*. produced a transgenic rat line that expresses rat prorenin in the liver, resulting in a 500-fold increase in circulating plasma prorenin levels (*36*). These rats had normal plasma renin levels and normal blood pressures, but developed severe cardiac hypertrophy. Similarly, Prescott *et al*. produced doubly transgenic mice expressing active human renin in the liver and human angiotensinogen in the heart (*37*). Because of the species-specificity of renin and angiotensinogen, Ang I would only be formed by human renin if it entered or were taken up by cardiomyocytes expressing human angiotensinogen. These mice had cardiac fibrosis accompanied by elevated cardiac Ang I and Ang II, but without changes in the circulating levels of these peptides. In another study, this group made doubly transgenic mice that expressed human prorenin in the liver and human angiotensinogen in the heart (*38*). These mice had elevated circulating levels of human prorenin, but no human angiotensinogen in plasma, and normal circulating plasma angiotensin levels and normal blood pressure. In the heart, there was an increase in Ang I, suggesting uptake and activity of human prorenin. However, there was no change in cardiac Ang II levels, and no physiological effects of Ang II on the heart, suggesting that the Ang I was generated in a compartment devoid of angiotensin converting enzyme (ACE). In other studies, mice overexpressing rat angiotensinogen in myocardium also had ventricular hypertrophy that was corrected by ACE inhibition and Ang II receptor blockade, although the plasma levels of Ang I and Ang II were unaltered (*39*).

Evidence of a Vascular Site of Renin Uptake

Renin activity was detected in the arterial wall as early as 1956 by Dengler (*40*), and later by Gould *et al*. (*41*). Indeed, several of the studies described above measured renin activity in the arterial wall (*25*, *28–30*) and showed that it correlated with plasma renin levels, and disappeared after nephrectomy. The site of renin uptake was also studied immunocytochemically (*27*, *42*, *43*). Following intravenous injection of mouse submandibular gland renin, renin immunoreactivity was detected predominantly in the media of the descending aorta and the central arteries of the spleen (*27*, *42*). Infusion of purified hog renin for several days into hypertensive rabbits resulted in uptake in the aorta, carotid artery and heart, and kidneys, mainly at the site of smooth muscle cells (*43*). Further indirect evidence of renin uptake into tissues was provided by studies using anti-renin antibodies that blocked its catalytic activity. In those studies, Ang II antagonists had a greater effect than anti-renin antibodies in reducing hypertension resulting from renin infusion (*23*), or Goldblatt two-kidney, one-clip hypertensive rats (*44*), suggesting that the location of the renin may not be accessible to the larger antibody molecule.

Biochemical and Molecular Evidence of Renin/Prorenin Binding

Renin angiotensin system (RAS) components may enter tissues in several ways. Measurements of tissue levels of such components and their comparison to plasma levels suggest that both can diffuse from the plasma into the interstitial spaces where angiotensin peptides are generated (*45*, *46*). Biochemical studies of isolated perfused beating hearts have further shown that angiotensin peptides can be formed in the interstitial spaces (*47*, *48*). These studies imply that renin and angiotensinogen may pass through the endothelial cell layer into the subendothelial spaces. This could occur by pressure-

mediated ultrafiltration through endothelial pores, as has been described in the glomerulus (*49–51*), or *via* other channels across endothelial membranes (*52*, *53*). However, the exact paths whereby renin and angiotensinogen enter tissues have not been determined.

In addition to transendothelial passage, several of the studies described above have provided evidence of renin uptake (*45*, *54*). We currently have knowledge of several renin- and prorenin-binding sites, which differ in their localization, specificity, and degree of characterization. Our present knowledge of these sites is discussed in the following section.

Renin Binding Protein (RnBP)

RnBP is a soluble renin binding protein (42,000 Da) that was originally identified in dog kidney (*5*). Yamamoto and coworkers observed that renin purified from the granule fraction of dog kidneys had a molecular weight of 43,000 Da, whereas renin purified from crude renal extract in the presence of thiol inhibitors had a molecular weight (MW) of 60,000 Da (*55–57*). Mixing of low MW renin from the granule fraction with a kidney cytosol fraction in the presence of sodium tetrathionate resulted in the formation of 60,000 Da renin (*5*).

RnBP was found in cortical tubular cells, but not in the glomeruli (*58*, *59*), and subcellular fractionation of rat kidney extracts revealed that most of the RnBP is contained in the cytosol (*60*). Recent studies using *in situ* hybridization to localize RnBP mRNA in rat kidney have yielded conflicting findings. One reported RnBP mRNA in the glomerulus (most likely in mesangial cells) (*61*), while another found RnBP mRNA in renal tubules (*62*).

Functionally, RnBP inhibits renin activity, which suggested that it might be involved in the regulation of RAS activity (*63*). However, because RnBP is a cytosolic protein and is therefore not secreted from the cell, it seems unlikely that it could be involved in the regulation of circulating RAS activity. Under normal conditions, renin probably does not come in contact with RnBP, at least in the plasma, and binding of renin to RnBP in cellular extracts may thus be an artifact of the extraction process. Leckie and coworkers found no changes in the renal levels of RnBP in the kidneys of rats with two-kidney one-clip hypertension, despite upregulation of renin expression in the clipped kidney (*62*). RnBP knockout mice, which completely lack the protein, have normal blood pressure, normal RAS activity, and respond normally to physiological stimuli that perturb the RAS. Taken together, these observations suggest that RnBP is not involved in the regulation of RAS activity or blood pressure.

RnBP Has *N*-Acyl-glucosamine, 2-epimerase Activity

RnBP has also been recognized as the enzyme *N*-acyl-glucosamine, 2-epimerase (NAGE), which catalyzes the intraconversion of *N*-acetyl-glucosamine (GlcNAc) and *N*-acetylmannosamine (*64*). Although RnBP/NAGE knockout mice have normal blood pressure, and normal excretion of *N*-acetyl-neuraminic acid (NANA—one of the principal metabolites of GlcNAc), they do excrete large amounts of an unknown oligosaccharide into the urine (*61*). In contrast, constitutive activation of the unidirectional UDP-dependent GlcNAc-2-epimerase causes developmental delay, and excessive NANA secretion (sialuria) (*65*). Recently, Bohlmeyer *et al*. reported an increase in RnBP in failing human hearts (*66*). Specifically, they observed redistribution in expression from microvascular endothelial cells to cardiac myocytes, and RnBP expression was highly correlated to pulmonary capillary wedge pressure among patients with end-stage idiopathic cardiomyopathy. Bohlmeyer *et al* (*66*). point out that RnBP/NAGE could be involved in modulating O-linkage of GlcNAc to cytoplasmic proteins by controlling the availability of GlcNAc (*67*). O-linkage of GlcNAc competes with phosphorylation of serine and threonine residues, is reversible, occurs with sufficient rapidity to serve a signaling/regulatory function, and has been shown to affect the activity and stability of several transcription factors and receptors (*68*). In the heart, O-linkage of GlcNAc might regulate alpha B-crystallin, which has been shown to play a cardioprotective role (*66*).

It is noteworthy that as RnBP/NAGE inhibits renin activity, so too renin inhibits NAGE activity (*69*). Therefore, it is possible that renin taken up into RnBP/NAGE-expressing cells might regulate NAGE activity. Although an earlier study found no evidence of changes in RnBP expression associated with changes in renal and circulating renin levels, the effects of RAS changes on the RnBP epimerase activities were not examined (*62*). More recently, a nonsecretable form of renin was identified (*70*, *71*). Peters and Clausmeyer demonstrated a four-fold increase in the mRNA in response to myocardial ischemia (*72*). This intracellular form of renin might also be involved in the regulation of NAGE activity.

Membrane-Associated Binding Sites

The cellular localization of RnBP suggests that it is unlikely to play a primary role in renin binding/uptake from plasma. However, earlier studies showed that although about 70% of kidney granule renin is released rapidly following isosmotic shock, 20% was released more slowly, and another 8% was retained in the insoluble fraction (*73*, *74*), suggesting that other membrane-associated renin binding sites might also be present.

Campbell and Valentijn (*6*) described a membrane binding site for ^{125}I-labeled renin that was most abundant in the mesenteric artery, and present at lower levels in the aorta, lung, and renal medulla, but not in the kidney cortex. However, they were unable to demonstrate saturable binding. Binding to mesenteric artery membranes was competed by the renin inhibitor SQ 30697, suggesting the involvement of

the active site in binding that would result in the inhibition of renin activity. Such a site would therefore be unlikely to contribute to the tissue generation of Ang I.

Using recombinant, metabolically labeled ^{35}S prorenin as a probe, Sealey and coworkers found evidence of a high affinity binding site in microsomal membranes of several tissues that bind both renin and prorenin (ProBP) (*7*). Relative binding was highest in the kidney, testes, and lung, lower in the brain, renal medulla, adrenal, heart, and aorta, and very low in skeletal muscle. The affinities for the renin and prorenin to binding sites in the preparations of renal cortex were in the 0.1–1.0 nmol/l range. The binding capacity of the renin/prorenin binding was around 200 fmol/mg membrane protein, which could bind sufficient renin to generate the excess Ang I in the range reported across the renal vasculature (*32*). Renin/prorenin binding was reversible, saturable, and pH- and temperature-dependent. The bound rat renin/prorenin was displaced by human renin/prorenin, but not by any of a variety of other potential ligands tested. Moreover, the binding did not appear to affect renin activity, nor did binding of prorenin result in its activation.

Mannose-6-phosphate (M6P)/Insulin-Like Growth Factor Receptor

More recently, Admiraal *et al*. reported uptake of renin/prorenin on endothelial cell membranes *via* the M6P receptor (*8*). This receptor binds proteins containing M6P, and also binds insulin-like growth factor II (IGFII) at an allosteric site. The M6P/IGFII receptor (R) has been shown to participate in the activation of latent transforming-growth factor β (TGFβ), possibly by inducing a conformational change that allows it to be cleaved by plasmin or other proteinases, or by creating a local environment with favorable kinetics (*e.g.*, high local concentrations of substrate and enzyme) (*75*). Binding to the M6P/IGFIIR leads to the activation of prorenin, and to the clearance of renin and prorenin (*8*, *76*, *77*). Local uptake of renin/prorenin may thus contribute to Ang II formation in the interstitial spaces of tissues, including those of the heart (*77*). Uptake of active renin into cells, or activation and uptake of prorenin, could provide a mechanism to generate Ang II intracellularly. Such a mechanism has been proposed to explain high intracellular levels of Ang II found in various tissues and has led to speculation concerning possible intracellular actions of Ang II (*78*, *79*). However, evidence for intracellular generation of Ang II could only be obtained when cells were transfected with a construct encoding a non-secretable form of angiotensinogen (*78*). Schuijt and Danser argued against the intracellular formation of Ang II, because of the absence of intracellular angiotensinogen and ACE (*80*), and the finding that incubation of cardiomyocytes in the presence of both prorenin and angiotensinogen failed to produce intracellular angiotensins (*81*). They concluded that the high intracellular concentrations of Ang II in tissues are more likely due to uptake and clearance of extracellular Ang II *via* the Ang II type 1 receptor (AT1R) (*80*). Nonetheless, the M6P/IGFIIR may mediate extracellular Ang II generation in the heart (*79*).

Another line of evidence suggests that uptake may occur independently of the M6P/IGFIIR. The TGR(mREN2)27 transgenic rat expresses the unglycosylated mouse *ren*-2^d gene under the control of its own promoter. This transgenic rat line has high levels of circulating unglycosylated prorenin, and develops cardiac hypertrophy (*82–84*). Peters and coworkers showed that adult cardiomyocytes can internalize unglycosylated prorenin and can generate angiotensins (*85*). In addition, hepatic expression of the *ren*-2^d under the control of an inducible promoter led to a marked rise in circulating and cardiac intracellular levels of unglycosylated renin and prorenin associated with hypertension, microinfarctions, necrosis, and inflammatory responses in cardiac tissue (*86*). However, the findings of such studies may depend on the species and the system used to express the RAS components and hence their cellular localization.

Expression Cloning of a Renin/Prorenin Receptor

Nguyen *et al*. used expression cloning to isolate a renin/prorenin receptor, although the method used to screen the cDNA library from which this clone was identified was not specified (*9*). They identified a human recombinant cDNA encoding a 350-amino acid protein with a single transmembrane domain that was expressed in the heart, brain and placenta, and at lower levels in the kidney and liver. The receptor was found in glomerular mesangial cells, as well as the subendothelium of renal and coronary arteries. Stably transfected cell lines expressing the receptor specifically bound renin and prorenin. Binding increased the catalytic activity of renin and appeared to activate prorenin, and also transduced an intracellular signal *via* extracellular signal-regulated kinase (ERK)1 and ERK2 mitogen-activated protein (MAP) kinases.

Further characterization of this site should include the preparation of knockout mice in which the gene encoding the receptor is inactivated. However, caution should be exercised in interpreting the findings of RAS studies in mice. Although mice have plasma renin activities and Ang I and Ang II levels similar to those of other species, mice achieve these levels very differently from most, if not all other species in which the RAS has been characterized. Mice have approximately 100-fold higher plasma renin, and correspondingly lower circulating angiotensinogen than other species (*87*, *88*). The excess renin in mouse plasma may require adaptation of any secondary system involving binding proteins or receptors. Other approaches to investigating the function(s) of this renin/prorenin receptor might involve overexpression in different tissues in mice and rats.

Implications of Renin/Prorenin Binding and Uptake

With the exception of the M6P/IGFIIR analyzed by Danser's

group, none of the membrane binding sites have been characterized beyond the initial reports. Only one novel specific receptor has been cloned (*9*), and the biological significance of this site remains to be determined. To date, no one has provided biochemical verification that any one of the renin/prorenin binding sites/receptors has a physiological function in the whole animal. However, there are a number of functions these sites could serve, which are discussed below.

Local Generation of Angiotensins

Most of the membrane-associated renin/prorenin binding sites that have been identified occur in relatively low abundance and are therefore difficult to isolate and study. Nonetheless, they may be present in sufficient quantities to explain the physiological differences in Ang I concentrations observed across vascular beds. In tissues that produce high levels of prorenin, renin binding may be displaced from receptors by prorenin. Binding to M6P/IGFII or renin/prorenin receptors might activate prorenin. In the case of the M6P/IGFIIR, prorenin is activated only after intracellular sequestration, and thus it is unlikely to contribute to the generation of extracellular Ang II. However, the renin/prorenin receptor appears both to increase the activity of bound renin, and to activate bound prorenin. This could lead to the generation of extracellular Ang II with local vasoconstrictor and vasculotoxic effects. If these functions of this receptor could be validated, the renin/prorenin receptor would be a potential target for anti-RAS drugs (*89*, *90*). Together, binding of renin/prorenin to one or a number of membrane sites could account for the excess angiotensins reported across vascular beds in physiological studies (*32*, *33*).

Buffering the Effects of Circulating Renin

Thurston and Swales originally proposed that renin in tissues maintains normal blood pressure (*26*). However, this need not exclude a role for the circulating RAS, which may respond to short-term changes induced by changes in posture, or hemorrhage. Organ systems may depend on renin uptake to maintain vascular tone, or other direct effects of Ang II or renin on tissues.

The blood flow requirements of different organs are determined primarily by their vascularity. However, Ang II receptor distribution and density could affect the sensitivity to Ang II, and the presence of a renin uptake mechanism, with the capacity to generate Ang II locally, could buffer fluctuations in circulating renin levels by integrating the plasma renin activity over time. The degradative functions proposed for the M6P/IGFIIR are also important to permit adaptation to circulating levels of RAS components. It is also conceivable that one receptor functions as the capture site where Ang I generation occurs, and another receptor acts to clear the bound renin. Such configurations would enable different vascular beds to adjust their sensitivity to fluctuations in renal renin release, and thus provide a mechanism to modulate the RAS effects in different organs ultimately regulated by a master control exerted at the level of renin released from the kidney.

Role in Prorenin Processing

In addition to its physiological role in mediating RAS in tissues, the M6P/IGFIIR provides a model for prohormone processing. Binding to this receptor has been shown to be required for cellular activation of latent TGFβ *via* proteolysis or other activating mechanisms (*75*). Similarly, prorenin could be secreted and then taken up by cells to be activated intracellularly as described by Danser and coworkers (*79*). Although the lack of intracellular angiotensinogen led them to conclude that this is most likely a degradative pathway, specialization of the lysosomal granules of juxtaglomerular cells might allow these cells to secrete active renin that is processed from prorenin secreted by those cells, and then taken up again. This is analogous to the processing of thyroglobulin which must be secreted for iodination to occur, but is then taken up again for conversion to T_3 and T_4 (*91*). It is also consistent with the correlation between circulating concentrations of renin and prorenin (*87*, *88*). Although, at this time, there is no evidence of the secretion of activated prorenin from endothelial cells where this possibility has been tested (*92*), the proteinase responsible for processing prorenin in smooth muscle cell-derived juxtaglomerular cells has yet to be identified, and perhaps models more complex than mere matching of an enzyme to a substrate will need to be evaluated (*93*, *94*).

Modulation of NAGE Activity—A Role in Diabetes

Renin uptake may also modulate RnBP/NAGE activity. In the glomerulus, the renin/prorenin receptor and RnBP/NAGE both appear to be expressed in mesangial cells. Thus, if the renin/prorenin receptor were also capable of translocating renin to the cytoplasm, it could be a mechanism to modulate NAGE activity. It is not known whether M6P/IGFIIR is present on mesangial cells, although it could also play a role at this site. In the heart, M6P/IGFIIR translocates renin into the myocytes where RnBP/NAGE is expressed under conditions of heart failure. Under these conditions renin uptake could reduce NAGE activity, resulting in either an increase or a decrease in GlcNAc, which could then affect intracellular signaling by altering the balance of O-linkage of GlcNAc/phosphorylation. It is especially intriguing that O-linkage of GlcNAc is affected by glucose concentration (*95*) and is involved in insulin signaling (*96*). RAS blockade is known to improve target organ damage in diabetic patients (*97*, *98*), and has been shown to improve insulin resistance in animal models (*99*). Although much of the benefit results from reduced AT1R stimulation, the addition of β-blockers to ACE inhibitor and diuretic therapy reduced the incidence of congestive

heart failure (*100*), and β-blockers alone appear to be beneficial in diabetic patients with congestive heart failure (*101*). These observations raise the possibility that plasma renin levels, which are reduced by β-blockers (*102*), may play a direct role, mediated by uptake *via* one or more of the receptor/binding sites and signal transduction *via* RnBP/NAGE.

Summary and Conclusions

There is considerable physiological evidence in support of renin uptake from the circulation and local generation of Ang II in tissues. A number of renin/prorenin binding sites have been identified. However, it is not known whether any of these sites mediate renin uptake *in vivo*. Each of the identified sites appears to have different biochemical characteristics, and several have not been further studied beyond the initial report. Thus, it remains possible that some of the less-well-characterized sites are the same. Among those sites, ProBP and the renin/prorenin receptor have the potential to bind renin and generate angiotensins locally. The renin/prorenin receptor might also activate prorenin, and transduce intracellular signals. However, further characterization is required. M6P/IGFIIR might also be involved in the local binding of renin/prorenin, and may further serve a clearance function. M6P/IGFIIR may also be involved in cycling prorenin between the Golgi, extracellular space, and lysosomal compartments of juxtaglomerular cells as part of a complex processing network similar to that involved in the conversion of thyroglobulin to thyroid hormones. However, there may be other receptors/binding sites involved, as suggested by transgenic studies of unglycosylated prorenin. Because RnBP/NAGE is localized intracellularly, it is unlikely to play a role in the local generation of Ang II. However, it may be involved in signal transduction because of its potential role in modulating GlcNAc levels, and hence in modulating O-linkage of GlcNAc to serine and threonine residues. Since M6P/IGFIIR and RnBP/NAGE may both be expressed in cardiomyocytes, together they may function to modulate cellular activities in failing heart cells in response to circulating renin and/or prorenin levels. Similarly, in the glomerulus, RnBP/NAGE may function together with the renin/prorenin receptor to modulate intracellular activity in response to renin and/or prorenin. Thus, more than one renin/prorenin binding protein/receptor may be required in mediating a physiological response. Further characterizations of these binding proteins and receptors and the biochemical and physiological functions they subserve are warranted.

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