Specific Prorenin / Renin Binding (ProBP)
Identification and Characterization of a Novel Membrane Site

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Renin can be detected in cardiovascular and other tissues but it disappears after bilateral nephrectomy indicating that tissues can take up or bind renal renin from the circulation. If renin uptake is the result of specific binding, plasma prorenin may be a natural antagonist of tissue directed renin-angiotensin systems.

To investigate if specific prorenin/renin uptake occurs in rat tissues, binding studies were performed with rat microsomal membrane preparations using recombinant rat prorenin metabolically labeled with 35S-methionine as a probe. A high affinity binding site for both renin and prorenin was identified. Affinities for prorenin and renin were approximately 200 and 900 pmol/L, respectively. Binding was reversible, saturable, and pH and temperature dependent. The relative binding capacities of membranes from various rat tissues were as follows (pmol/mg): renal cortex (55), liver (54), testis (63), lung (31), brain (18), renal medulla (15), adrenal (17), aorta (7), heart (4), and skeletal muscle (1).

Bound prorenin was displaced by rat and human renin or prorenin but not by the prosequence of rat prorenin, angiotensin I or II, rat or human angiotensinogen, the renin inhibitor SQ30697, atrial natriuretic factor, amylase, insulin, bovine serum albumin, hemoglobin, heparin, lysozyme, ovalbumin, cytochrome C, pepsin, pepsinogen, ribonuclease A, mannose-6-phosphate, a-methyl mannoside, gonadotropin releasing hormone, or an antibody to hog renin binding protein.

These results demonstrate specific binding of prorenin to a site in rat tissues, herein named ProBP, that also binds renin. It is possible that differences in prorenin/renin binding capacity determine the activity of tissue-directed renin-angiotensin systems and that prorenin is a natural antagonist. Alternatively, a prorenin/renin receptor may have been identified that may function by transducing an intracellular signal.

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Because prorenin appears to have highly localized effects, we proposed that it might bind to tissues.\textsuperscript{10}

Circulating renin may also bind to tissues. Infused renin is taken up by vascular tissues.\textsuperscript{11,12} Renin has been extracted from cardiovascular tissues from intact,\textsuperscript{13,14} but not from bilaterally nephrectomized,\textsuperscript{15} rats, indicating that the source of renin in the vasculature is the kidneys. When they discovered renin, Tigarstedt and Bergman\textsuperscript{19} observed that the pressor effects of renal extracts last for only minutes in intact rabbits, but for hours in bilaterally nephrectomized rabbits. Such rabbits might have vacant binding sites for renin. Other studies suggest that tissue-directed renin might be catalytically active.\textsuperscript{17} The evidence for regulated uptake of renin has been strengthened by the recent demonstration of binding of extrinsically labeled \textsuperscript{125}I-renin to membranes from rat mesenteric arteries.\textsuperscript{18} It is not known if prorenin binds to the same site.

By binding to the same site as renin, prorenin would be a natural antagonist of tissue directed plasma renin-angiotensin systems. Such an effect might explain the association of the high prorenin levels of pregnancy and diabetes mellitus with vasodilation\textsuperscript{19,20} and the positive relationship between prolonged exposure to high prorenin levels in teenage diabetic patients and hyperperfusion injury.\textsuperscript{19,20}

In this study we searched for evidence of prorenin and renin binding in a variety of rat tissues. To avoid potential artifacts, intrinsically labeled recombinant rat prorenin was used. To avoid underestimation of the binding capacity of the various tissues, endogenous renin was removed from all membrane preparations.

**MATERIALS AND METHODS**

**Buffers**

\textbf{Buffer I for Renin Assay} This buffer was 50 mmol/L Tris-Cl, pH 7.4, containing 0.1 mol/L NaCl, 0.5% bovine serum albumin (BSA), 0.1% EDTA, and 0.1% Na\textsubscript{2}SO\textsubscript{4}. This buffer was incubated at 56°C for 0.5 h to denature any contaminants in the BSA, and stored at 4°C.

\textbf{Buffer II for Prorenin Activation} This buffer was 0.5 mol/L Tris-Cl, pH 7.5, 5 mmol/L CaCl\textsubscript{2}, and 1% BSA.

\textbf{Buffer III for Membrane Preparation and Dilution} This buffer was 10 mmol/L Tris-Cl, pH 7.5, containing 10 mmol/L MgCl\textsubscript{2}, 1 mmol/L KCl, 0.1% EDTA, and 0.1% Na\textsubscript{2}SO\textsubscript{4}.

\textbf{Buffer IV} This was buffer III with the addition of 0.3 mol/L sucrose.

\textbf{Buffer V for Dilation of Ligands} This buffer was 50 mmol/L phosphate, pH 7.5, 0.5% BSA, 0.1% EDTA, 0.1% Na\textsubscript{2}SO\textsubscript{4}, 0.05% phenylmethylsulfonyl fluoride (PMSF), and 0.05% 8-OH-quinoline.

\textbf{Buffer VI for Dialysis of Prorenin} This buffer was 50 mmol/L sodium phosphate, pH 7.5, 100 mmol/L NaCl, 0.1% EDTA, and 0.1% Na\textsubscript{2}SO\textsubscript{4}.

\textbf{Buffer H for Prorenin Purification} This buffer was 50 mmol/L Tris-Cl, pH 8.0 containing 0.1 mol/L NaCl, 0.1% EDTA, 0.1% Na\textsubscript{2}SO\textsubscript{4}, and 10 mmol/L benzamidine.

**Preparation of Rat Renin Secreting GH\textsubscript{4} Cells (GH\textsubscript{4}R)** Rat renin cDNA coding sequences\textsuperscript{16} were amplified by polymerase chain reaction (PCR) using the primers 5'-GGAGACATCATGGCGAGGAGGATG-3' and 5'-GATCCCTAGGGCCAAAGGCAAA-3'. The amplified DNA fragment was inserted into the Smal site of the expression vector pEE14. The resulting rat renin (rREN) expression vector was transfected into a GH\textsubscript{4} cell line.\textsuperscript{22} Although the vector pEE14 contains a selectable marker for glutamine synthetase resistance,\textsuperscript{23} it proved difficult to maintain stable transformants using methionine sulfonamide (the antibiotic that is degraded by glutamine synthetase). Therefore, cells were cotransfected with pSV-Neo and selected for G418 resistance as described by Chu et al.\textsuperscript{22} Transformants were maintained in the presence of 200 \(\mu\)g/mL G418.

The clone selected for production and labeling of prorenin (GH\textsubscript{4}R) produced at least 50,000 ng angiotensin 1 (AI)/mL/h per 24 h (0.5 \(\mu\)g prorenin/mL) when started at about 50% confluence in fresh Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS).

**Preparation of Recombinant Rat Prorenin** The final product of renin gene expression in GH\textsubscript{4}R cells is prorenin. For large-scale purification, cells were maintained at, or near confluence in 175 cm\textsuperscript{2} flasks containing DMEM plus 10% FBS. Media were cleared of cell debris by centrifugation at 2,700 \(\times\) g for 10 min at 20°C.

For the production of \textsuperscript{35}S-prorenin, labeling was performed using cells that were plated 24 h beforehand, and grown to 40% to 50% confluence in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. The cells were then rinsed with methionine-free DMEM containing 10% dialyzed FBS and incubated in this medium for 3 h. The medium was replaced with DMEM containing 10% dialyzed FBS, 5 mCi \textsuperscript{35}S-methionine (100 Ci/mmol) and 3 \(\mu\)g/mL cold methionine (10% of the usual concentration). After 18 h incubation, the medium was collected and centrifuged to remove cell debris. Free \textsuperscript{35}S-methionine was removed by ultralfiltration using a 2.2 mL Amicon centricron (YM30), adding 3 \(\times\) 2 mL aliquots of Dulbecco's phosphate buffered saline (DBS) and reducing the volume 10-fold after each addition.

**Preparation of an Anti-Rat Prorenin Prosequence IgG Affinity Column** The 46-amino acid prosequence of rat prorenin [CSFSLPDITA5FGRIILKKSMSVRHELEERGVDTRISAEGFRIK] was synthesized (Im-
munodynamics, Menlo Park, CA) and used to inoculate rabbits (Immunodynamics). IgG from rabbit IR 657 was purified by affinity chromatography with Protein A Sepharose according to recommendations of the manufacturer (Pharmacia, Uppsala, Sweden). The purified IgG was then coupled to CNBr-activated Sepharose again following the manufacturer’s recommendations (Pharmacia). An affinity precolumn was prepared by omitting the antibody from the coupling reaction.

Affinity Purification of Recombinant Rat Prorenin Nonradioactive Prorenin Medium from GH4R cells was precipitated with 65% ammonium sulfate to concentrate the starting material 20- to 50-fold. The precipitate was against buffer H and loaded onto the 100 mL CNBr precolumn without antibody described above. The precolumn flow-through, which contained prorenin, was loaded at 0.1 mL/min directly onto a 100 mL immunoaffinity column and the flow-through was monitored at A280. The immunoaffinity column was washed with buffer H until the A280 was either zero or constant (approximately 10 bed volumes). Prorenin was then eluted with 0.2 mol/L glycine, pH 3.0. The fractions with the highest prorenin concentration were pooled, dialyzed against buffer VI and then stored at –40°C. Pure rat renin was generated from prorenin by trypsin activation as described below under “Measurement of Prorenin and Renin.”

35S-Labeled Prorenin and Renin The ammonium sulfate step was omitted. For purifications using the anti-rat prosequence antibody, cell culture medium was diluted 1:4 (v:v) with buffer H and then loaded onto a 10 mL precolumn. The precolumn effluent was loaded directly onto the immunoaffinity column. The precolumn was disconnected, and the affinity column was washed with buffer H until the A280 was either zero or constant. The fractions with the highest prorenin concentration were pooled, dialyzed against buffer VI, and then stored at –40°C.

Measurement of Prorenin and Renin Prorenin was converted to renin during incubation with trypsin as follows. To 10 µL sample were added to 90 µL buffer I and 20 µL TPCK-treated trypsin (3.0 mg/mL in buffer II) (Sigma) and the mixture incubated at room temperature for 30 min. The reaction was stopped by adding 30 µL soybean trypsin inhibitor (5 mg/mL in 0.1 mol/L NaCl). The renin activity so formed was measured by an enzyme kinetic assay followed by radioimmunoassay (RIA) of the angiotensin I, as follows: to 120 µL of the activated sample were added, 100 µL buffer I, 2 µL TPCK treated trypsin (3.0 mg/mL), 2 µL 5% PMSF, 2 µL 5.7% 8-hydroxyquinoline (angiotensinase inhibitors) and 35 to 100 µL nephrectomized rat plasma (a source of angiotensinogen to provide a final concentration of 1 µg AI equivalents/mL). The sample was incubated for 1 h at 0°C and 37°C. The generated AI was measured by RIA. The results are expressed as nanograms AI/milliliters/hour after subtraction of the AI in the sample incubated at 0°C.

Intrinsic renin activity was determined in samples that were treated similarly but not exposed to trypsin. Prorenin was calculated as the difference between the renin activities in the trypsin and nontrypsin activated samples.

Gel Electrophoresis of Recombinant Rat Prorenin Rat prorenin was subjected to 12% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (BioRad, Richmond, CA, or Novex, San Diego, CA, gel apparatus with precast gels) and transferred to a nitrocellulose membrane. Prorenin was detected by Western blot with the rabbit anti-rat prorenin prosequence antibody (IR657) and alkaline phosphatase-labeled goat anti-rabbit IgG by silver staining. Two dimensional electrophoresis of 38S-labeled prorenin was performed with minitube gels (Biorad) for isoelectric focusing (IEF) and the second dimension was performed with a 12% slab gel. Deglycosylation of N-linked carbohydrate was performed with PNGaseF and EndoH (New England Biolabs, Beverly, MA) using 1250 units of enzyme per sample and buffers provided by the manufacturer.

Membrane Preparation Unanesthetized 200 to 300 g normal rats were decapitated. Their organs were removed, washed with iced saline, weighed, and frozen at –40°C. Unless otherwise stated, all steps were performed at 4°C. Ice chilled buffer IV, 4 mL/g wet tissue, was added. Tissue was homogenized on ice for 30 sec (twice at speed 10 and once at speed 6; Polytron, Brinkmann Instruments, Westbury, NY) and centrifuged at 2,000 g for 30 min. The supernatant was centrifuged at 15,000 g for 30 min. The 15,000 g supernatant was then centrifuged again at 100,000 g for 70 min. The 100,000 g pellet was suspended in 4 volumes of buffer III per original gram of tissue and centrifuged again at 100,000 g.
To remove renin from the membranes, the pellet was incubated for 1 h on ice with 1 volume of 4 mol/L MgCl₂. Finally, the membranes were washed twice with 5 volumes of buffer III and centrifuged at 100,000g. If residual renin activity remained higher than 10 ng AI/h/50 µg protein, membranes were reextracted with 4 mol/L MgCl₂. Membranes were stored at -40°C. Protein concentration was determined by Lowry assay using BSA as the standard.

**Binding Assays** At first, the binding assay was performed in 5 mL tubes in a total volume of 300 µL. Later, to conserve on membranes and ligand, the volume was reduced to 150 µL (identical concentrations) and incubated in 1.5 mL microcentrifuge tubes. Either 100 µL or 50 µL aliquots each of membranes (50 µg or 25 µg microsomal membrane protein in buffer III), radioligand (70 to 100 pmol/L in buffer V), and, if appropriate, competitor ligand were mixed gently on ice. Tubes were then incubated at 4°C for 18 h to reach equilibrium. Nonspecific binding (NSB) was defined by the addition of 10⁻⁶ mol/L prorenin (final concentration) or 3 × 10⁻⁸ mol/L renin. In pilot NSB experiments, purified prorenin was compared to ammonium sulfate precipitated crude prorenin. Identical results were obtained. Therefore, unless stated otherwise, ammonium sulfate precipitated prorenin or renin was used to determine nonspecific binding. The binding assay was terminated by adding 2 mL (300 µL assay) or 1 mL (150 µL assay) ice chilled buffer III followed by centrifugation at 2800 rpm, 4°C, for 20 min (300 µL assay), or in a tabletop microfuge at 12,000 rpm for 5 min (150 µL assay). The wash and centrifugation step were repeated once. The pellet was resuspended in 20 µL 4 mol/L urea and counted in a Packard scintillation counter after adding 5 mL Atomlight scintillation fluid (Dupont-NEN, Boston, MA). Data were analyzed by the weighted nonlinear least squares modeling methods of DeLean and Lefkowitz. Dissociation constants were averaged as geometric means and ranges were calculated.

*The ligands for competition binding experiments were purified rat renin or prorenin, partially activated human prorenin (43% prorenin, 57% renin), the generous gift of Dr. Robert Heinrikson (Upjohn, Kalamazoo, MI), rat, and human angiotensinogen, the generous gift of Dr. Robert Heinrikson (Upjohn, Kalamazoo, MI).* Rat renin was not present in the rat prorenin preparation.

**Preparation of Prorenin** Purified rat prorenin electrophoresed on a 12% SDS polyacrylamide gel as a doublet composed of a major band of M₁, approximately 48,000 and a minor band of slightly higher molecular weight (Figure 1A). ³⁵S-Methionine-labeled rat prorenin also displayed a similar doublet (Figure 1B). When deglycosylated with the mannose-specific N-linked endoglycosidase EndoH or the nonspecific endoglycosidase PNGaseF, prorenin appeared to decrease in molecular weight on an SDS gel and to lose the upper faint band (Figure 1B). This result provides strong evidence that the GH4 cell prorenin is a mannose-containing glycoprotein similar to CHO cell human prorenin. Two dimensional gel electrophoresis of the labeled material further resolved five major and two minor discrete species, suggesting varying levels of posttranslational modification (Figure 1C), a result that has previously been observed for endogenous renin by others. Enzymatic specific activity of the purified rat prorenin, determined by measuring the enzymatic activity of a known mass of trypsin-cleaved prorenin in the presence of 1 µmol/L angiotensinogen (usually 1 µg of...
prorenin by Coomassie staining) averaged 20 ng AI/h/ng prorenin, similar to published values.\(^{40}\)

**Binding Studies With \(^{35}\)S Rat Prorenin** Initial experiments showed that \(^{35}\)S-prorenin bound specifically to rat renal cortical membranes. An incubation temperature of 0\(^\circ\) to 4\(^\circ\)C was used to avoid potential proteolytic effects and to decrease nonspecific binding, which increased above 4\(^\circ\)C (Figure 2). The pH optimum for binding was between 7.0 and 7.5. There was an abrupt fall in binding below pH 6.0, and a more gradual decline above pH 7.5. Pretreatment of membranes at 56\(^\circ\)C caused a sharp decrease in prorenin binding to less than 10% of control values with both renal cortex (10.1 ± 0.51 fmol/mg) and liver membranes (5.3 ± 0.58 fmol/mg). Our studies revealed a very slow association rate at 0\(^\circ\) to 4\(^\circ\)C of approximately 15 h at 100 pmol/L ligand and 30 pmol/L receptor concentration. Off-rate kinetics displayed a half-life greater than 24 h at 0\(^\circ\)C.

**A Survey of Prorenin Binding to Various Tissues** To survey differences in the tissue binding, microsomal membranes were prepared from a variety of rat tissues, and binding experiments were performed at a fixed, nonsaturating concentration of \(^{35}\)S prorenin (70 pmol/L). Specific binding was measured for each membrane source together with membrane protein concentration. As shown in Figure 3, there were differences in binding between tissues. Relative binding was highest in the renal cortex (55 fmol/mg), liver (54 fmol/mg), and testes 63 (fmol/mg), intermediate in lung (31 fmol/mg), brain (18 fmol/mg), renal medulla (15 fmol/mg), and adrenal gland (17 fmol/mg), low in aorta (7 fmol/mg) and heart (4 fmol/mg), and very low in skeletal muscle (1 fmol/mg). Although these measurements are a function of both the binding affinity and the number of sites available for binding, the affinities of the sites in liver, kidney and heart are similar (see below). Therefore, these measurements...
TABLE 1. BINDING AFFINITIES AND CAPACITIES FOR RENAL CORTEX, LIVER, AND HEART

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Renal Cortex (n = 7)</th>
<th>Liver (n = 2)</th>
<th>Heart (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$, pmol/L</td>
<td>220</td>
<td>270</td>
<td>160</td>
</tr>
<tr>
<td>$B_{max}$, fmol/mg</td>
<td>216</td>
<td>220</td>
<td>39</td>
</tr>
</tbody>
</table>

prorenin (Figure 7). Mathematical modeling of the experimental results suggested that a two-compartment model best fit the data. $V_1$ averaged 643 mL/kg (range 554 to 712 mL/kg) in three nephrectomized rats and 476 mL/kg in an intact rat. The total apparent volume of distribution ($V_d$) averaged 1132 (range: 988 to 1404) mL/kg and 924 mL/kg, respectively.

**DISCUSSION**

In the present study we identify a high affinity binding site for renin and prorenin in membranes from rat tissues. We propose to name the site ProBP. Binding was highly specific. Bound prorenin was displaced only by related proteins (rat prorenin > rat renin > human prorenin) while numerous other potential ligands had no effect. Binding capacity was highest in renal cortex, liver, and testes, intermediate in lung, brain, adrenal gland, and renal medulla, low in heart and aorta, and almost undetectable in skeletal muscle.

The demonstration of a specific binding site for renin supports the results of many investigators who have suggested that renin is taken up by tissues. The results indicate that renin may be actively taken up by both renal and extrarenal tissues and that the extent of binding differs markedly between organs.

Although our studies did not specifically address the question of whether renin is active when bound, renin activity was consistently detected in renal membrane preparations and could be removed only by treatment with 4 mol/L MgCl$_2$. It is therefore likely that renin is active when bound. A tissue binding site may serve as a buffer for acute changes in renin, acting as a renin reservoir, or may be a new locus at which blood pressure may be regulated. Tissue binding of renin and localized angiotensin production might explain why spontaneously hypertensive rats (SHR) respond to blockade of the renin system despite having normal circulating renin levels. Renal renin binding may also explain why a dose of an angiotensin antagonist that is too low to have an effect when infused into the peripheral circulation lowers systemic BP in the SHR when infused intrarenally.

These results also provide the first evidence for specific prorenin binding. They are consistent with our hypothesis that prorenin has a localized effect. The observation that prorenin is inactive when bound may explain prorenin's putative vasodilator action since occupancy of binding sites by inactive prorenin would diminish renin uptake thereby limiting the capacity of tissue-bound renin to generate the vasoconstrictor hormone angiotensin II. This interpretation is consistent with the premise that prorenin is a natural antagonist of tissue-directed renin-angiotensin systems. Alternatively, we may have identified a prorenin/renin receptor that, when activated, may transduce an as yet unidentified intracellular event.

The distribution of binding is consistent with our

**FIGURE 5.** Competition of prorenin binding to rat kidney microsomal membranes. **A.** Competition experiments with rat $^{35}$S-prorenin and unlabeled rat prorenin and renin. Rat renin was prepared by activation of prorenin with trypsin followed by pepstatin affinity chromatography. **B.** Competition experiments with rat $^{35}$S-prorenin and rat prorenin or human prorenin/renin (43% prorenin/57% renin).
knowledge concerning prorenin. Infusion of prorenin is associated with only minor falls in blood pressure, indicating that renin and prorenin binding are not likely to be high in resistance vessels. The very low degree of binding to aorta and skeletal muscle is consistent with that premise. High prorenin levels are associated with increased blood flow to kidneys and reproductive organs, consistent with the high levels of binding we found in kidneys and testes. High adrenal binding is also not unexpected. Like the kidneys and testes, the adrenal glands express the prorenin gene and transgenic rats with excessive adrenal renin gene expression secrete large amounts of prorenin and adrenal steroids into the circulation, raising the intriguing possibility that adrenal prorenin/renin uptake may have effects on steroid production.

Preliminary studies reported herein demonstrated a high volume of distribution of exogenously administered recombinant prorenin in rats (greater than the extracellular fluid volume) indicating that significant amounts of renin and prorenin may be sequestered in organs with high binding capacities. Hiruma et al similarly observed a very high volume of distribution for infused rat renal renin. The prorenin/renin binding capacity of renal cortex is 200 fmol/mg membranal protein, the yield of which was close to 1% of the tissue mass. Therefore, a renal cortex of 0.5 g (0.5 mL), containing approximately 5 mg of membranes, is likely to have 1000 fmol (2 nmol/L) binding sites. A plasma renin concentration of 5 ng/mL/h with 4-fold more prorenin than renin would result in a combined renin and prorenin concentration of 5 pmol/L. A binding site with a Kd of 200 pmol/L would be no more than 2.5% occupied, 2% by prorenin and 0.5% by renin, meaning that it would contain in the range of 5 fmol or 25 ng AI/h of renin activity. This would result in tissue renin levels of 10 fmol/mL (50 ng/mL/h), five times the normal circulating concentration. In the vicinity of the glomerulus, where renin and prorenin are secreted, the renin concentration is likely to be much higher than in blood. Such renal renin binding may help to explain the very high levels of angiotensin II that have been measured in renal cortical compartments.

There are marked differences in the binding site described herein and those reported by Campbell and Valentijn, which may be explained by differences in experimental design or by the identification of a different class of binding sites. Campbell and Valen-
Decay curves of plasma prorenin after injection of 400 ng prorenin into intact (open symbols) or nephrectomized (solid symbols) 250 g rats. Baseline plasma prorenin was subtracted from the reported prorenin levels.

Prorenin is not a ligand (Personal communication, Dr. Miyake). RnBP mRNA is undetectable in liver extracts, a site at which we observed high levels of specific binding. To distinguish the factor identified herein from RnBP we propose to use the acronym ProBP.

Binding sites have been demonstrated for other circulating enzymes and proenzymes, e.g., thrombin and plasminogen. In some instances, as with plasminogen, binding is not associated with an intracellular event. In others, as with thrombin, the enzyme cleaves a portion of the binding site, which then leads to the transduction of a signal. So far we have no evidence that prorenin/renin binding transduces a secondary intracellular or membranal event. Multiple possible responses could potentially occur following prorenin/renin binding. These include second messenger production, internalization and processing of the ligand, receptor endocytosis, and desensitization.

CONCLUSIONS

In summary, specific binding of renin has been demonstrated in rat tissues. Since the same site also binds prorenin we have termed it ProBP to distinguish it from RnBP. ProBP binding capacity differs between tissues indicating that prorenin/renin uptake may be regulated differently in various organs and that changes in binding could determine the activity of putative tissue renin angiotensin systems. These results also identify a role for prorenin. By competing for binding, or by transducing an intracellular signal, prorenin may be the natural antagonist of tissue-directed renin-angiotensin systems. That would explain the association of high prorenin levels with renal vasodilation and its presence in reproductive organs, which maintain extraordinarily high blood flows. It might also explain the development of diabetic vascular disease subsequent to hyperperfusion injury, which occurs in patients with high plasma prorenin levels.

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