Regulated Systemic Activation of Rat Plasma Prorenin

GREGORY M. T. HARE, PETROS IOANNOU, STANISLAW DUBISKI, and DANIEL H. OSMOND

SUMMARY Nephrectomized rats have above-normal plasma prorenin levels, presumably of extrarenal origin, but essentially no renin, suggesting a lack of "convertase" for prorenin activation. Adrenalectomized rats have low plasma prorenin levels accompanied by high renin activity, suggesting enhanced prorenin activation by the action of a stimulated "convertase" mechanism. Cross-circulation between adrenalectomized and nephrectomized rats for 15 or 30 minutes, dramatically lowered prorenin and raised renin levels in both types of rats, suggesting extensive activation of prorenin to renin. Similarly, in vitro mixing of these bloods (without cross-circulation) raised renin activity over five times the expected calculated level, while prorenin essentially disappeared. In both cases, prorenin from nephrectomized rat plasma apparently was activated to renin by the enhanced action of "convertase" in the adrenalectomized rat plasma. This newly generated renin activity was, like normal plasma renin, almost completely inhibited by a monoclonal antibody against hog renin and generated an immunoreactive angiotensin I. In contrast, cross-circulation or in vitro mixing of blood from normal control and nephrectomized rats produced little detectable activation of prorenin and only modest increments of renin, suggesting relative inactivity of the "convertase" mechanism in normal plasma. Our data suggest that activation of plasma prorenin is a significant regulated pathway for renin production, as it is greatly stimulated after adrenalectomy and deficient after nephrectomy, thereby implicating the kidney as an important contributor to the "convertase" mechanism operating within the circulation. (Hypertension 10: 122-126, 1987)

KEY WORDS: prorenin • activation • rat • nephrectomy • adrenalectomy

TRAditionally, all renin activity in the blood was thought to originate from the kidneys by direct secretion of active renin, the quantity of which was regulated to provide appropriate angiotensin II levels required for fluid, electrolyte, and blood pressure control. It is now recognized that the renin precursor prorenin is abundantly present in the blood of humans\textsuperscript{1-4} and animals,\textsuperscript{5-10} and is therefore implicated as a potentially important source of additional plasma renin, assuming the existence of a regulated "convertase" mechanism for activating it.

If prorenin in the blood is indeed activated, then plasma renin activity (PRA) could represent the sum of renin secreted from the kidney and renin derived by activation of plasma prorenin. It follows that the regulation of these two sources of renin would have to be synchronized: conditions in the body that promote renal renin release should also stimulate prorenin activation in the blood. However, the proportion of PRA derived by renal renin secretion and plasma prorenin activation has yet to be determined.

Despite the potential importance of prorenin "convertase," little is known about it. Several blood enzymes reportedly participate in the activation of prorenin in vitro,\textsuperscript{11,12} but it cannot yet be assumed that all are similarly involved in vivo, separately or together, as members of a multienzymic cascade. Recent evidence suggests that a renal enzyme (or enzymes) is of special importance,\textsuperscript{13} notably because the extrarenal prorenin present in plasma after nephrectomy in dogs\textsuperscript{13,14} and rats\textsuperscript{15,16} apparently is not converted to renin in the body. Thus, there are putative enzymes, systemic, or renal, or both, that could be part of a "convertase" mechanism, but the operation of this mechanism in vivo is not well established and certainly not defined.

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The purpose of this study was to establish more clearly the existence and regulation of prorenin activation in the blood. Blood expected to be normal or high in prorenin "convertase" (from control and adrenalectomized rats, respectively) was mixed in vitro with blood postulated to be low in "convertase" (from nephrectomized rats) in order to determine the effects on prorenin activation. Similar mixing was also produced in vivo by cross-circulation. Our data support the hypothesis of a regulated systemic conversion of prorenin to renin, the efficacy of the "convertase" involved in terms of renin production from extrarenal prorenin, and the antigenic cross-reactivity of this new renin generated from prorenin with normal renin.

Materials and Methods
Cross-circulation Experiments
Cross-circulation experiments were performed in male Wistar rats weighing 200 to 300 g (Charles River Canada, St-Constant, Quebec, Canada) with the rats under sodium ethyl-(1-methyl-propyl)-malonyl-thiourea anesthesia (Inactin; Promonta, Hamburg, Federal Republic of Germany). Rats were paired by weight, the right carotid artery and left jugular vein of each animal were cannulated with polyethylene cannulas (PE-90, Intramedic; Clay Adams, Parsippany, NJ, USA), and heparin, 1000 U/kg (Hepalean; Organon Canada Ltd., West Hill, Ontario, Canada), was administered. The average weights of animals paired for autocirculation or cross-circulation were within 5% of each other. Two experimental protocols were used: one in which control rats were paired with nephrectomized (2AX) rats and the other in which adrenalectomized (2NX) rats were paired with 2NX rats. The nephrectomies and adrenalectomies were performed with the rats under halothane anesthesia (Fluothane; Ayerst Laboratories, Toronto, Ontario, Canada) approximately 24 and 48 hours prior to cross-circulation, respectively.

Within each protocol three separate groups of paired animals (n = 5 pairs each) were used for: 1) autocirculation of paired rats in which blood from the carotid artery was circulated to the jugular vein of the same rat (no cross-circulation), 2) cross-circulation of paired rats for 15 minutes, and 3) cross-circulation of a different set of paired rats for 30 minutes. An Ohaus Harvard Trip Balance (Ohaus Scale Corp., Union, NJ, USA) was used to maintain constant blood volume of cross-circulated rats, as described by Sommenberg et al. The hematocrit values were determined for each animal before and after autocirculation or cross-circulation. At the end of each circulation period, all animals were exsanguinated from the carotid artery and the blood was placed in test tubes containing EDTA, 4.5 mg/ml blood. In rats with kidneys, the renal blood vessels were clamped immediately before exsanguination. An aliquot of blood from each partner of the autocirculated pairs was mixed 1:1 at 4°C, in vitro, to determine the effect of mixing. The theoretically expected values for renin and prorenin after mixing were calculated from the renin and prorenin values of separate plasmas before mixing, with correction for the relative plasma volumes caused by differences in hematocrits of the two types of blood sampled.

Determination of Plasma Prorenin and Renin Activity
Prorenin and renin activity were determined as described previously1 and adapted to rat plasma.10 Prorenin was activated by incubating plasma with trypsin (4 mg/ml, Sigma T-8253, 11,680 BAEE U/mg) for 60 minutes at 23°C, after which the reaction was stopped by adding LBTI (6 mg/ml, Sigma T-9378, inhibitor capacity, 15,000 BAEE U/mg). Angiotensin generated for 10 minutes at 37°C, pH 6.0, was determined by radioimmunoassay (Dupont-New England Nuclear, Medical Diagnostics Division, North Billerica, MA, USA) and the PRA was expressed in terms of nanograms of angiotensin I (ANG I) generated per milliliter per hour of incubation. Prorenin was calculated as the difference between control (unactivated PRA) and the PRA obtained after tryptic activation.

Monoclonal Renal Antibody
The hybridoma cell line F-32 VIII C4, developed by Dorer et al.18 to produce antibodies against hog renal renin was obtained from the American Type Culture Collection (Rockville, MD, USA) and successfully cultured. Then, 5 x 10⁶ cells were injected intraperitoneally into BALB/cJ mice (Jackson Laboratories, Bar Harbor, ME, USA) primed with pristane (Sigma T-7640). A comparable booster injection was given 2 to 3 weeks later. Antibody was purified from the resulting ascites fluid by Affi-Gel protein A affinity chromatography (MAPS II kit; Bio-Rad Laboratories, Richmond, CA, USA). The final preparation was equilibrated in 0.1 M Tris buffer, pH 7.4, at a concentration of 1.2 mg/ml. Serial dilutions of antibody (1:500 to 1:10) incubated with rat plasma (1:1) for 18 hours at 4°C revealed that a 1:10 dilution of antibody completely inhibited PRA in normal rat plasma (Table 1).

Statistical Analysis
Statistical significance was determined using a non-paired t test in all cases and a two-tailed distribution of t values.

Results
Autocirculation
The autocirculation data served as the baselines against which to compare the corresponding cross-circulation data. Plasma from normal control rats contained both renin and prorenin, while 2NX rats had about four times as much prorenin and essentially no renin (Figure 1). Plasma from 2AX rats had renin activities about four times higher than the normal control value, with essentially no prorenin (Figure 2). The 2NX rats again had high prorenin levels with virtually no renin.

The theoretically expected values for renin and prorenin.
TABLE 1. Inhibition of Rat Plasma Renin with Monoclonal Anti-renin

<table>
<thead>
<tr>
<th>Antibody dilution</th>
<th>PRA (ng ANG I/ml/hr)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0±0.3</td>
<td>—</td>
</tr>
<tr>
<td>1:500</td>
<td>2.8±0.3</td>
<td>60</td>
</tr>
<tr>
<td>1:250</td>
<td>1.8±0.2</td>
<td>74</td>
</tr>
<tr>
<td>1:100</td>
<td>0.5±0.3</td>
<td>92</td>
</tr>
<tr>
<td>1:50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1:10</td>
<td>0</td>
<td>100</td>
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PRA values are means ± SEM (four determinations on a normal rat plasma pool).

renin after proportional (1:1) mixing of either normal with 2NX blood or 2AX with 2NX blood was calculated, after correcting for the hematocrits of each blood sample (see Materials and Methods and Figures 1 and 2). The open bars in Figures 1 and 2 represent the expected levels of renin and prorenin after mixing. The actual values obtained from plasma after mixing blood either in vitro, after autotoculation, or in vivo, by cross-circulation, were compared with these expected arithmetic means to determine whether anything other than pure mixing occurred (i.e., prorenin activation.) In vitro mixing of normal and 2NX blood after autotoculation (no cross-circulation) produced plasma prorenin and renin values close to the corresponding calculated averages. The actual prorenin value in mixed plasma was statistically similar to the calculated value, while the actual renin level was slightly higher (see Figure 1). Similar in vitro mixing of 2AX and 2NX plasma produced dramatically different results. After mixing, prorenin disappeared while renin levels increased over five times above the expected calculated level (see Figure 2). This huge increase in PRA, presumably generated from prorenin, was 97% inhibited by our anti-renin antibody.

Cross-circulation

Hematocrit values of the paired rats became identical within 15 minutes of cross-circulation and remained so at 30 minutes in all pairs. There were no significant differences in the renin or prorenin values obtained from the partners of paired cross-circulated rats.

Cross-circulation of normal control and 2NX animals for 15 and 30 minutes tended to increase prorenin levels in both rats above the expected calculated mean, but not above values found in 2NX rats (see Figure 1). Renin levels rose progressively during 15 and 30 minutes of cross-circulation to a maximum level of 49.9 ± 15.8 ng ANG I/ml/hr above the expected mean. On the other hand, cross-circulation of 2AX and 2NX animals produced distinctly different results. Prorenin levels dropped significantly after both 15 and 30 minutes of cross-circulation when compared with either the expected theoretical mean (see Figure 2) or the corresponding normal-2NX cross-circulation levels (p<0.05 in all pairs; see Figure 1). PRA rose significantly after 15 and 30 minutes of cross-circulation when compared with either the theoretical mean (see Figure 2) or the normal-2NX cross-circulation levels (p<0.05 in all pairs; see Figure 1). PRA increased by a maximum of 328.5 ± 102.6 ng ANG I/ml/hr.

Discussion

We interpret the present data as follows. Normal control plasma contains both renin and prorenin, while 2NX plasma contains above-normal (extrarenal) prorenin and virtually no active renin (see Figure 1). This finding confirms earlier data in dogs^{13} and rats^{15, 16} and supports our hypothesis and observations that an important renal "convertase" of prorenin disappears after nephrectomy.^{10} Thus, 2NX plasma is low in renin and "convertase."
Proportional mixing of normal control and 2NX blood in vitro produced a plasma prorenin value that was close to the arithmetic average of the two separate prorenin values; there was little indication that "convertase" in the control plasma had activated any prorenin in the 2NX plasma (shown by the black bar in Figure 1). The renin value of the mixture was also close to the expected mean after mixing. Surprisingly, cross-circulation for 15 and 30 minutes raised the prorenin level above the expected mean, but not above the level found in 2NX rat plasma, while PRA increased only slightly (see Figure 1). Thus, prorenin contributed by the 2NX rats apparently raised the prorenin level of the control rats without much conversion of it to renin on either side.

In contrast, plasma from 2AX rats had very high renin and low prorenin values relative to control animals, suggesting a very high endogenous "convertase" activity. Essentially all the prorenin had already been activated to renin, with little remaining to be activated by trypsin (see Figure 2). Mixing 2AX and 2NX blood in vitro produced a rapid interaction: virtually all the prorenin in 2NX plasma appeared to be activated to renin, resulting in a PRA over five times above the expected value (shown by the black bar in Figure 2). Similar results were obtained from blood mixed in vivo by cross-circulation for 15 and 30 minutes, indicating that prorenin activation had occurred rapidly within the circulation. In other words, the endogenous "convertase" in the 2AX blood was great enough to activate most of the extrarenal prorenin circulating in the 2NX rats, indicating its efficacy upon such prorenin.

Monoclonal antibody against hog renin blocked 90% of the renin activity in 2AX plasma and 97% of the new renin produced by mixing 2AX and 2NX blood. Thus, renin generated by the action of 2AX "convertase" on extrarenal prorenin in 2NX blood was immunologically indistinguishable from active renin in normal or 2AX rat plasma.

These data point to the existence of a powerful, regulated, "convertase" mechanism for the activation of blood prorenin. This "convertase" is much more active in 2AX rats than in normal control rats, suggesting that the high renin state of adrenalectomy is also a high "convertase" state. Thus, conversion of plasma prorenin could be a second pathway for the production of active renin that is regulated in concert with renal renin release, but the relative importance of each pathway is not yet defined. Our data further support the view that plasma prorenin is a functionally integrated component of the circulating renin-angiotensin system.

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