Release of Active and Inactive Renin by the Porcine Kidney

Michael D. Bailie, Franz M.H. Derkx, and Maarten A.D.H. Schalekamp

SUMMARY We studied the relative rates of release of active and inactive renin by the kidney in anesthetized pigs. Renin concentration was determined in arterial and renal venous plasma as follows: (1) before and after stimulation of renin release with isoproterenol or furosemide, (2) after suppression of renin release by extracellular fluid volume expansion, and (3) after administration of propranolol or indomethacin. Inactive renin was activated by dialysis of plasma at pH 3.3 for 24 hours. Renin concentration was estimated by radioimmunoassay determination of angiotensin I after a 3-hour incubation with excess homologous renin substrate. Following isoproterenol, the release of active renin increased from 8 ± 4 (SEM) to 58 ± 34 ng/min, and inactive renin increased from 53 ± 33 to 321 ± 136 ng/min. Similarly, furosemide stimulated the release of both active and inactive renin. Both forms of renin were suppressed by propranolol or indomethacin. Although changes in renin release following volume expansion were not statistically significant, the direction of change for both forms of renin was similar. Following logarithmic conversion of the rate of release, the plot of active vs. inactive renin formed a straight line. Values for active renin as a percentage of the total renin in simultaneously drawn arterial and renal venous plasma samples were not different. Thus, under the conditions of these experiments, release of active and inactive renin appears to be controlled by similar mechanisms. Both stimulation and suppression of renin release result in parallel changes in release of the two forms. Data on relative amounts of active renin in arterial and renal venous plasma suggest that there is no systemic conversion of the two forms. Circ Res 44: 32-37, 1979

RECENT REPORTS suggest the presence of an inactive form of renin in plasma and in renal tissue of both normotensive and hypertensive patients (Derkx et al., 1976; Weinberger et al., 1977; Day et al., 1975; Sealey et al., 1976). Studies from several laboratories have centered on that form of renin which is inactive at physiological pH but activated by treatment at acid pH (Derkx et al., 1976; Weinberger et al., 1977). This acid-activated or inactive renin* has also been reported to be present in several species in addition to man (Boyd, 1974; Rubin, 1972; Leckie, 1973; deSenarclens et al., 1977; James and Hall, 1974; Skeggs et al., 1967). Whether inactive renin represents a true proenzyme or renin bound to an inhibitor remains unclear (Leckie and McConnell, 1975). It also has been suggested that inactive renin may be the intrarenal storage form of the enzyme (deSenarclens et al., 1977).

Acid-activated renin has been measured in systemic and renal venous plasma of man under varying conditions (Derkx et al., 1976; Weinberger et al., 1977). These data suggest that changes in the concentration of active and inactive renin in plasma may be dissociated under certain circumstances. However, no data are available relating the release of active and inactive renin in experimental animals. Therefore, studies were carried out to determine the rate of release of both forms of renin by the porcine kidney. The pig was selected as the experimental animal because acid-activated renin has been isolated from its kidney (Boyd, 1974; Rubin, 1972). In addition, inactive renin has been reported to be released by the isolated perfused pig kidney (Boyd, 1974). To correlate the rate of release of active and inactive renin, renin release was stimulated with either isoproterenol or furosemide, and suppressed by volume expansion, propranolol, or indomethacin. By varying widely the rate of release of renin, we could better observe the relationship between release of the active and inactive forms.

Methods

Surgical Procedures

Experiments were carried out on pigs 45–57 days old weighing 3.0–13.5 kg. These pigs were small enough to study easily but still had mature renal function (Gruskin et al., 1970). Anesthesia was induced with intraperitoneal pentobarbital sodium (35 mg/kg). Additional anesthetic was given during the experiment as required. The pigs were intubated and ventilated (Loosko Amsterdam Infant Ventilator). Rectal temperature was monitored and body temperature maintained with a heating pad and...
Radiant heat lamp. A catheter was placed in the femoral artery to obtain arterial blood samples and to measure blood pressure using a strain gauge transducer. A catheter was placed in the external jugular vein for infusion of saline and drugs. A second catheter was placed in the bladder via a small midline abdominal incision.

The left kidney was exposed through a retroperitoneal flank incision and the left ureter cannulated. The renal artery and vein were carefully dissected free from the surrounding tissue, and a noncannulating electromagnetic flowmeter probe (Transflow 500 Electromagnetic Bloodflow Meter) was placed on the renal artery. A curved 22-gauge needle attached to a polyethylene catheter was inserted into the renal artery for infusion of saline or isoproterenol. A period of 45–60 minutes was allowed for stabilization following completion of surgery.

**Experimental Protocols**

**Effects of Isoproterenol, Furosemide, Propranolol, and Indomethacin**

The following protocol was observed in six pigs. During a control period, two sets of arterial and renal venous blood samples were obtained 5 minutes apart. An infusion of a solution of isoproterenol (1.0 μg/ml per kg) was started into the renal artery at a rate of 0.1 ml/min with a Braun infusion pump. The infusion rate was increased gradually until renal blood flow increased. After 5–7 minutes, two more sets of arterial and renal venous blood samples were obtained 5 minutes apart. The infusion of isoproterenol then was stopped, and following a 45–60 minute recovery period a third collection of urine and two more blood samples were obtained.

Following recovery from the isoproterenol, the pigs were given furosemide, 1 mg/kg, iv. As urine flow increased, isotonic saline containing KCl (4.5 mEq/liter) was infused into the jugular vein at a rate equal to urinary output. Ten minutes after the diuretic was given, two sets of arterial and renal venous blood samples were collected 5 minutes apart. A second injection of furosemide, 2 mg/kg, was given and the sampling repeated.

The effect of two inhibitors of renin secretion then was determined. Immediately after sampling, following the second dose of furosemide, propranolol, 1 mg/kg, was given intravenously, and after 10 minutes two sets of blood samples were obtained. Indomethacin, 2 mg/kg, was given intravenously and a final set of blood samples obtained.

**Effects of Volume Expansion, Furosemide, and Indomethacin**

In five pigs the response to volume expansion and furosemide was determined. After control blood samples were obtained, the pigs were given isotonic saline equivalent to 5% body weight, infused over 30 minutes. The infusion rate then was adjusted to equal urine flow rate. When an apparent steady state urine output was reached, two sets of arterial and renal venous blood samples were drawn 5 minutes apart. The pigs were then given furosemide, 2 mg/kg per hr, as a constant intravenous infusion and the saline infusion increased to match urine flow rate. When urinary output had stabilized, blood samples again were obtained. The pigs then received indomethacin, 2 mg/kg, and a final set of samples was drawn.

**Analytical Techniques and Data Handling**

All blood samples were collected in tubes chilled on ice and containing disodium ethylenediaminetetraacetic acid (EDTA, 1.8 mg/ml). Blood samples were centrifuged within 5 minutes of collection and the plasma removed. The red blood cells then were reconstituted to the original hematocrit in saline and returned to the pig. Plasma for renin determinations was stored at −20°C until assayed. Hematocrit was determined by the micro method.

Renin in arterial and renal venous plasma was estimated as described by Derkx et al. (1976). Plasma was dialyzed at either pH 4.5 or 3.3 for 24 hours at 4°C and then returned to pH 7.4 by dialysis for another 24 hours. After dialysis, plasma was incubated with excess homologous renin substrate, and the angiotensin I generated was determined by radioimmunoassay. The renin concentration of plasma dialyzed at pH 4.5 was active renin. Following dialysis at pH 3.3, the concentration of renin was greater, representing the sum of the active and acid-activated renin, i.e., total renin. Inactive renin was calculated as the difference between total renin and active renin. Renin substrate was prepared according to the method of Skinner (1967). The substrate was found to be free of renin and angiotensinase activity. Incubation of substrate with renin resulted in zero order kinetics. The concentration of renin in plasma was expressed as nanograms of angiotensin I generated per milliliter per hour (ng/ml). Renin release was calculated as the product of the venous-arterial concentration difference and the renal plasma flow. Mean arterial blood pressure, heart rate, and mean renal blood flow were taken directly from digital readout.

Statistical analysis was carried out using analysis of variance and t-test for paired and unpaired comparisons. The level of significance was set at 5%. Because significant heterogeneity of variance in renin release existed, all data were converted to logarithms prior to statistical analysis.

**Results**

**Effects of Isoproterenol, Furosemide, Propranolol, and Indomethacin**

The release of total renin increased following intrarenal arterial infusion of isoproterenol (Fig. 1).
This increase in the release of total renin was due to an increase in the rate of release of both active and inactive renin. During infusion of isoproterenol, renal blood flow increased from 73 ± 10 to 94 ± 6 ml/min, whereas mean systemic blood pressure decreased from 114 ± 3 to 107 ± 4 mm Hg. Both changes were significant.

Following the first dose of furosemide (1 mg/kg) there was an increase in the release of total renin from the kidney (Fig. 2). As with isoproterenol, the increase was due to changes in the release of both active and inactive renin. Although renal blood flow increased in each pig, the change from 87 ± 8 to 95 ± 9 ml/min was not statistically significant. No consistent changes in mean arterial pressure were noted.

The second injection of furosemide (2 mg/kg) did not result in any further increase in the release of total, active, or inactive renin (Fig. 2). No significant changes in hemodynamics were noted, although blood flow averaged 102 ± 9 ml/min after the second dose of diuretic.

The systemic administration of propranolol resulted in a significant decrease in the release of total renin (Fig. 3). This change again was accompanied by a significant change in both the active and inactive forms of the enzyme.

The administration of indomethacin produced a further fall in the rate of release in total renin from 104 ± 47 to 37 ± 20 ng/min. Active renin decreased from 32 ± 9 to 9 ± 3 ng/min, and inactive renin decreased from 74 ± 47 to 39 ± 15. Although these changes were not statistically significant, they were seen in five of the six pigs.

The effects of isoproterenol, furosemide, propranolol, and indomethacin on renal blood flow, and the concentration of renin in renal venous plasma are shown in Table 1. Although each drug tended to cause a change in blood flow, the concentration of renin in renal venous plasma increased with isoproterenol and furosemide and decreased with propranolol and indomethacin.

**Effects of Volume Expansion, Furosemide, and Indomethacin**

In this group of pigs, the treatments resulted in changes in release of total, active, and inactive renin (Table 2). However, because of the wide variation among animals, the changes were not statistically significant.

**Relation of Release of Active and Inactive Renin**

When the logarithm of the rate of release of active and inactive renin was plotted, the relative proportions of the two forms of the enzyme remained constant (Figs. 4 and 5). This relationship appears to hold regardless of the treatment. This finding is supported by the data demonstrating that the relative concentration of active renin in either arterial or renal venous plasma is not changed by...
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Table 2  Renin Release (ng/min) under Control Conditions and following Saline Volume Expansion, Furosemide, and Propranolol

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Saline</th>
<th>Furosemide</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>121 (57)</td>
<td>77 (42)</td>
<td>1035 (664)</td>
<td>621 (342)</td>
</tr>
<tr>
<td>Active</td>
<td>23 (6)</td>
<td>14 (9)</td>
<td>190 (87)</td>
<td>47 (47)</td>
</tr>
<tr>
<td>Inactive</td>
<td>98 (44)</td>
<td>57 (38)</td>
<td>937 (538)</td>
<td>574 (334)</td>
</tr>
</tbody>
</table>

Values are mean (SEM in parentheses); n = 5.

Discussion

An acid-activated form of the enzyme renin has been demonstrated in kidneys of several animal species including the pig (Boyd, 1974; Rubin, 1972; Leckie, 1973; deSenarclens et al., 1977; Skeggs et al., 1967). The studies of Leckie and McConnell (1975) suggest that inactive renin may be coupled to an inhibitor, which is destroyed by acidification. Most studies on experimental animals to date have concentrated on the estimation of tissue renin content, although James and Hall (1974) did measure acid-activated renin in dog plasma.

Boyd (1974) has demonstrated that porcine kidney contains at least two forms of renin. Renin A had a molecular weight of 40,000 and was more active than renin B, which had a molecular weight of 60,000. Renin B could be converted to renin A by acidification. Furthermore, he demonstrated stimulation of release of renin A and B in response to isoproterenol in the isolated perfused kidney. Therefore, it appeared reasonable to carry out experiments in the anesthetized pig.

In the present studies, the plasma concentration and release by the kidney of two forms of renin were determined. Several authors have demonstrated that renin is activated by dialysis of human plasma at pH 2–3 (Derkx et al., 1976; Weinberger et al., 1977), and by dialysis of renin extracted from pig and rabbit kidneys at similar pH (Boyd, 1974; Rubin, 1972; Leckie, 1973). In the studies reported here, a comparison of plasma renin concentration at pH values above 4.5 to concentrations at 4.5 and 3.3 was not made. However, in view of the reported data and the low concentration of active renin in

Table 1  Renal Blood Flow (RBF) and Renal Venous Renin (RVR) Concentration in Pigs during Control Periods (C) and following Isoproterenol (ISO), Furosemide (F), Propranolol (P), and Indomethacin (IM)

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>ISO</th>
<th>C</th>
<th>F-1</th>
<th>F-2</th>
<th>P</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF (ml/min)</td>
<td>73 (10)</td>
<td>94 (6)</td>
<td>87 (8)</td>
<td>95 (9)</td>
<td>102 (9)</td>
<td>85 (8)</td>
<td>75 (7)</td>
</tr>
<tr>
<td>T-RVR (ng/ml)</td>
<td>4.8 (0.4)</td>
<td>12.3 (4.9)</td>
<td>4.2 (1.0)</td>
<td>14.1 (3.0)</td>
<td>19.2 (3.6)</td>
<td>9.2 (1.4)</td>
<td>4.4 (5.9)</td>
</tr>
<tr>
<td>A-RVR (ng/ml)</td>
<td>0.8 (0.1)</td>
<td>1.9 (0.8)</td>
<td>0.8 (0.2)</td>
<td>2.0 (0.5)</td>
<td>3.3 (0.8)</td>
<td>1.6 (0.3)</td>
<td>0.8 (0.2)</td>
</tr>
<tr>
<td>I-RVR (ng/ml)</td>
<td>4.1 (0.3)</td>
<td>10.3 (3.7)</td>
<td>3.4 (0.8)</td>
<td>12.9 (2.8)</td>
<td>14.9 (3.4)</td>
<td>7.6 (1.3)</td>
<td>3.6 (0.5)</td>
</tr>
</tbody>
</table>

Values are mean (SEM in parentheses); n = 5. F-1 = furosemide, 1 mg/kg; F-2 = furosemide, 2 mg/kg; T = total renin, A = active renin, I = inactive renin.
The relation between the release of active and inactive renin in pigs during control periods (Δ) and receiving isoproterenol (●), furosemide (○), propranolol (Ο), and indomethacin (Χ). Correlation coefficient = 0.82; n = 5.

The relation between the release of active and inactive renin in pigs during control periods (Δ) and treated with saline volume expansion (Ο), furosemide (●), and indomethacin (Χ). Correlation coefficient = 0.77; n = 5.

Table 3

Percent Active Renin in Arterial and Renal Venous Plasma

<table>
<thead>
<tr>
<th></th>
<th>Active</th>
<th>Isoproterenol</th>
<th>Furosemide</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>19.6</td>
<td>16.6</td>
<td>16.1</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>(3.9)</td>
<td>(2.5)</td>
<td>(2.5)</td>
<td>(5.3)</td>
</tr>
<tr>
<td>Renal</td>
<td>16.7</td>
<td>14.4</td>
<td>17.4</td>
<td>19.5</td>
</tr>
<tr>
<td>venous</td>
<td>(0.9)</td>
<td>(1.7)</td>
<td>(2.3)</td>
<td>(2.6)</td>
</tr>
</tbody>
</table>

Values are mean (SEM in parentheses), n = 5.

inactive forms of the enzyme (Fig. 1 and 2), whereas suppression of release caused both to decrease (Fig. 3). Determination of renin release over a wide range of values indicates a consistent relationship between the release of active and inactive renin under the conditions of these studies (Figs. 4 and 5). Furthermore, the change in concentrations of both active and inactive renin in renal venous plasma indicates that the effects of the drugs on renin release were at least partially independent of renal blood flow (Table 1).
eral venous plasma (Weinberger et al., 1977). The nature of the mechanisms which could possibly lead to interconversion of the two forms of renin are unknown. The amount of intrarenal renin present in the inactive form may be related to a balance between synthesis and release (deSenarclens et al., 1977). However, the reason that the inactive form is released and is not all converted first to active renin is unknown. Further physiological and biochemical studies will be required to clarify these issues specifically.

A fourth possible source for the differences between the results of these studies and those obtained for man may be related to the interconversion of active and inactive renin in plasma and/or the relative rates of metabolism of the two forms of the enzyme. The failure to find any change in the relative concentration of active renin in simultaneously obtained arterial and renal venous plasma samples (Table 2) suggests that there is no major interconversion of the two forms in plasma. However, changes could be masked by differences in the rate of catabolism of the two forms of the enzyme.

In the present studies the biochemical differences in the forms of renin were not determined. Although it has been demonstrated by several authors that acidification appears to activate an inactive form of renin (Derkx et al., 1976; Weinberger et al., 1977; Day et al., 1975; Boyd, 1974; Leckie, 1973), there is still confusion concerning the molecular nature of the product. Until further biochemical clarification is available, the possibility remains that the activated enzyme may not be renin but may act nonspecifically on angiotensinogen to form angiotensin I (Haber and Slater, 1977).

We conclude from these studies that both the active and acid-activated form of inactive renin are released from the kidney of the pig. The mechanisms controlling release of both forms of the enzyme in this animal model appear to be similar. The physiological significance of inactive renin remains obscure, and additional studies will be required to clarify its importance in regulation of the renin-angiotensin system.

Acknowledgments

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