Dissociation of Circulating Renin and Erythropoietin in Rats

By R. M. Donati, M.D., J. J. Bourgoignie, M.D., C. Kuhn, M.D.,
N. I. Gallagher, M.D., and H. M. Perry, Jr., M.D.

With the Assistance of Linda Kurz, B.A.

ABSTRACT

Renin and erythropoietin activities were measured in the plasma of 110 rats exposed to various conditions designed to dissociate the two renal "hormones." In acute experiments, intraperitoneal injection of polythiazide increased renin activity but did not change erythropoietin activity; exposure to low oxygen tension increased erythropoietin activity and caused an insignificant decrease in renin activity. In chronic experiments, salt restriction or the combination of salt loading and desoxycorticosterone for 2 weeks increased or decreased renin activity, respectively, without changing erythropoietin activity. The combination of chronic salt loading and acute hypoxia reduced renin activity and increased erythropoietin activity. In the final experiment, intraperitoneally injected cobaltous chloride acutely increased erythropoietin activity without markedly altering renin activity. Without contributing to the solution of the problem of the cell origin of erythropoietin, the present study clearly indicates that the secretion of renin and erythropoietin can be dissociated.

ADDITIONAL KEY WORDS

cobalt  polythiazide  sodium balance  hypoxia

The juxtaglomerular cell of the kidney has been suggested as the site of origin of both renin and erythropoietin. Evidence for the secretion of renin by juxtaglomerular cells is considerable (1, 2). Evidence that erythropoietin is secreted by these cells is much less convincing and has been inferred almost entirely from studies in which alterations of renal or circulating erythropoietin have been associated with changes in granulation of the cells (3). The methods used to increase secretion of erythropoietin might well have simultaneously increased renin production and thereby increased changes in juxtaglomerular cell granulation. This possibility, which must be excluded before any definite relationship can be established between erythropoietin secretion and juxtaglomerular cell granulation, was not tested by measuring renin.

Our experiments have no bearing on the problem of the cell of origin of erythropoietin. They do bear on the question whether the secretion rates of erythropoietin and renin can be altered independently. Thus far, renin and erythropoietin activities have been simultaneously measured only rarely, and in these instances there has generally been an increase in both (4, 5). Here we have measured the levels of renin and erythropoietin activity in the plasma of rats exposed to experimental conditions which were chosen in an attempt to dissociate the two renal "hormones." Increased renin activity was induced by rendering the sodium balance negative, either rapidly with diuretics or more slowly with restriction of dietary sodium; decreased
### TABLE 1

Rerun and Erythropoietin Activity in Rat Plasma

<table>
<thead>
<tr>
<th></th>
<th>No. rats</th>
<th>Renin activity (mg/100 ml plasma)</th>
<th>Erythropoietin activity % Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Acute Hypoxia or Polythiazide Administration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular diet</td>
<td>23</td>
<td>829 ± 67</td>
<td>0.88 ± 0.43</td>
</tr>
<tr>
<td>Regular diet plus polythiazide</td>
<td>14</td>
<td>222 ± 193*</td>
<td>0.90 ± 0.28</td>
</tr>
<tr>
<td>Regular diet plus hypoxia</td>
<td>21</td>
<td>691 ± 87</td>
<td>13.80 ± 2.93*</td>
</tr>
<tr>
<td>B. Chronic Sodium Restriction or Loading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular diet</td>
<td>10</td>
<td>749 ± 79</td>
<td>0.44 ± 0.48</td>
</tr>
<tr>
<td>Sodium restriction</td>
<td>13</td>
<td>1250 ± 100*</td>
<td>0.45 ± 0.27</td>
</tr>
<tr>
<td>2% saline and DCA</td>
<td>9</td>
<td>152 ± 20*</td>
<td>0.60 ± 0.10</td>
</tr>
<tr>
<td>C. Acute Cobalt Administration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 μmoles Co²⁺</td>
<td>5</td>
<td>1630 ± 89</td>
<td>0.84 ± 0.23</td>
</tr>
<tr>
<td>50 μmoles Co²⁺</td>
<td>5</td>
<td>954 ± 107</td>
<td>0.13 ± 1.11†</td>
</tr>
</tbody>
</table>

Figures are ±SEM. Part A gives results from five experiments, part B from two experiments, and part C from one experiment. See Methods for description of the experimental conditions.  
*P < 0.025. †P < 0.005. ‡P < 0.001.

---

Renin activity was induced by feeding excess salt and simultaneously injecting desoxycorticosterone acetate (DCA). Either hypoxia or administration of cobalt was used to stimulate erythropoietin activity.

### Methods

A total of 110 female rats of the Sprague-Dawley strain, each weighing about 200 g, was used in three types of experiments; the numbers of animals used in each type are indicated in parts A, B, and C of the table. For the first type, the rats were given Purina Laboratory Chow, which contained 0.275 g of iron and 90 g of sodium/kg of food, and tap water, both ad libitum. Some rats received 0.5 ml of 0.9% saline solution intraperitoneally at 0 and 12 hours; others received 1 mg of polythiazide (Renese, Pfizer) in 0.5 ml saline solution by the same route and at the same times. Twelve hours after the second injection approximately half of the group receiving the regular diet were given tap water to drink; the rest were given 2% saline solution and in addition received 0.5 mg DCA every 2 days by intraperitoneal injection. The experiment continued for 2 weeks, after which blood was collected from all animals. A second experiment had the same design except that some animals were kept at a pressure of 0.5 atm for the final 18 hours; these hypoxic animals are included in Figure 1 but not in the table.

The last type of experiment involved ten rats fed Purina Laboratory Chow and tap water; they

---

1Table 1 includes only 100 animals; the other 10 were hypoxic rats used in the second type of experiment and are included in Figure 1.
received a single intraperitoneal injection of either 5 or 50 μmoles of cobaltous chloride 12 hours before blood was collected.

Blood obtained by cardiac puncture was collected in heparinized syringes and immediately centrifuged for 10 minutes at 1200 x g. The plasma was separated and frozen. Renin and erythropoietin activities were measured as follows.

Renin activity was determined in plasma that was dialyzed and incubated by a modification of Helmer's method (6) and assayed in the nephrectomized, pentolinium-treated rat (7). Each sample of plasma was tested four times in three different animals, and each pressor response was closely bracketed with the pressor responses from known amounts of synthetic 1-L-asparaginyl 5-L-valyl angiotensin II (hypertensin, Ciba). Renin activity is expressed as milli-micrograms of angiotensin II equivalents present in 100 ml of plasma after 1 hour of incubation at 37°C and pH 5.7.

Erythropoietin activity was measured by a modification of Cotes and Bangham's method (8), using red blood cell incorporation of radioiron in plethoric, ex-hypoxic mice as an indirect determination of erythropoietin activity. The plasmas from all animals in each experiment were pooled and assayed in at least five mice. The results are expressed as percent of injected radioiron incorporated 72 hours after intraperitoneal injection of 0.4 ml of untreated test plasma and are reported in percent of 56Fe.

Results

Within 32 hours, 1 mg of intraperitoneally injected polythiazide increased renin activity in the plasma of 14 rats (P < 0.001) without significantly changing erythropoietin activity. Exposure to 0.5 atm for 8 hours increased the erythropoietin activity in the plasma of 21 rats (P < 0.001) with a slight but insignificant decrease in renin activity (Table 1, part A).

The plasma of 13 rats that had essentially no salt in their diet for 2 weeks had increased renin activity (P < 0.025). In contrast, 9 rats that had 2% saline to drink for 2 weeks and in addition received 0.5 mg DCA by intraperitoneal injection every 2 days, had decreased renin activity in their plasma (P < 0.001). In neither group of rats was there a simultaneous demonstrable change in erythropoietin activity (Table 1, part B). The combination of chronic salt loading and acute hypoxia produced simultaneous reduction in renin activity and increase in erythropoietin activity (Fig 1).

Within 12 hours, 50 μmoles of intraperitoneally injected cobaltous chloride increased erythropoietin activity in the plasma of 5 rats (P < 0.005) without markedly altering renin activity. Neither erythropoietin nor renin activity was significantly altered by 5 μmoles of cobalt (Table 1, part C).

Discussion

It has been suggested that erythropoietin is produced by juxtaglomerular cells, and hence that its secretion into the plasma may be related to that of renin, which is almost certainly produced there. The evidence that erythropoietin is present in juxtaglomerular cells is indirect and unconvincing (3). Takaku and co-workers (9) attempted to correlate the granularity of juxtaglomerular cells with the intensity of the erythropoietic response in rats. They found increased circulating erythropoietin activity and increased juxtaglomerular granulation following renal arterial constriction, hemorrhage, or phenylhydrazine-induced hemolytic anemia; conversely, suppression of erythropoiesis by hypertransfusion resulted in diminished granulation. Unfortunately, each of these stimuli could alter renin activity and thereby alter juxtaglomerular granulation. Either decreased renal perfusion pressure or hemorrhage has been found to increase circulating renin levels (10), and acute hemolytic anemia might be expected to have a similar effect by decreasing circulating blood volume. On the other hand, hypertransfusion has been reported to decrease renal renin (11). In rats briefly exposed to hypoxia, increased erythropoietin activity was said to be accompanied by increased juxtaglomerular granulation in one report (12) but not in another (13). Following 2 to 6 weeks of hypoxia, Demopoulos and co-workers (12) and Oliver and Brody (14) demonstrated increases in granulation with concomitant increase in erythropoietin activity or hematocrit. In these experiments, hyponatremia, hypotension, or hypovolemia, factors which might have stimulated renin production, were absent; however, renin ac-
tivity was not measured. Using rabbits and dogs, other investigators confirmed the increase in circulating erythropoietin activity following constriction of a renal artery (15-17). Although increases in both renin and erythropoietin activities in canine (5) and human (4) plasma have been associated with renal infarction, Abbrecht and co-workers (5) have clearly shown that the releases of renin and erythropoietin were not simultaneous and that renin appeared first. Increases in the activities of both "hormones" have also been observed in patients with renal arterial stenosis (4).

Without helping to resolve the problem of the cell of origin of erythropoietin, the present study has clearly shown that the secretion of renin and erythropoietin into the peripheral plasma of rats can be dissociated and that the activity of one of these "hormones" can be altered without a concomitant alteration in the activity of the other. By changing sodium balance, circulating renin activity could be significantly increased or decreased without affecting erythropoietin activity. In contrast, acute hypoxia or cobalt greatly increased circulating erythropoietin without significantly altering renin activity. Hypoxia would be expected to increase erythropoietin activity as its primary and major effect; it might also be expected to have a secondary and minor effect of decreasing renin activity in view of the increase in blood volume which Demopoulos (12) ascribed to it.

Acknowledgments


References

16. Fischer, J. W., Schonfield, R., and Porteous,

Dissociation of Circulating Renin and Erythropoietin in Rats
R. M. DONATI, J. J. BOURGOIGNIE, C. KUHN, N. I. GALLAGHER, H. M. PERRY, Jr. and Linda Kurz

doi: 10.1161/01.RES.22.1.91

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1968 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/22/1/91

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/