# Renin in Anesthetized Rats (37460)

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Several hypotheses concerning the control of renin release by the kidney are based on observations made using rats (1–5), an animal in which renal renin release has never been directly quantitated. The data lacking are measurements of renal plasma flow and renal venous minus arterial renin activities. In the experiments described below, presently available methods were adapted for use to obtain some of the lacking data.

*Methods.* Albino rats of both sexes, whose weights averaged 352 ± 10 g were used for these experiments. They were kept in a constant-temperature room and had continuous access to tap water and lab chow. Sodium pentobarbital, 45 mg/kg body weight, administered into the tail vein, was used as the anesthetic. A jugular and a cartotid were catheterized with polyethylene tubing (PE-50) and a tracheotomy was performed. Through an abdominal midline incision, the left ureter was catheterized using PE-50 previously stretched to form a fine tip. The left renal vein was catheterized as follows, using a length of PE-50 the tip of which was stretched to reduce its diameter and make it more flexible. Two ties were threaded around the right ileolumbar vein, one of which was used to ligate the vein. The tubing was inserted through a slit in the vein made proximal to the tie. The catheter was then threaded into and up the vena cava until its tip could be manipulated into the renal vein. After positioning the tip 3–4 mm from the kidney, the second tie was tightened around the ileolumbar vein and the catheter. The incision and abdominal contents were covered with gauze moistened with isotonic NaCl.

Following surgery, a prime (10 $\mu$Ci $^3$H-PAH, para-aminohippuric acid, dissolved in a small volume of isotonic NaCl) was given and a sustaining intravenous infusion (0.36 $\mu$Ci/min $^3$H-PAH in isotonic NaCl) was started. The infusion rate was 0.05 ml/min. After a 45-min equilibration period, a 45-min clearance was begun. Arterial and renal venous bloods were sampled at the midpoint. Two consecutive 45-min clearances, separated by a 45-min period, were performed in a group of 16 rats. For a group of 6 rats, the rate of infusion was increased from 0.05 to 0.2–0.5 ml/min, in proportion to body weight, after the first clearance; after a 45-min wait, a second 45-min clearance period was begun.

Arterial and renal venous blood samples, about 0.5 ml each, were slowly withdrawn into ice cold plastic syringes and centrifuged at once in a cold room; 0.2 ml of plasma was pipetted into small polyethylene tubes which were kept on ice, while 0.01 ml 1.2 *N* HCl was mixed in. Thirty minutes later, 0.01 ml of 0.6 *N* NaOH was mixed in. This procedure irreversibly inactivates a renin inactivator found in rat plasma (6). The tubes were frozen until incubation with substrate could be completed.

Aliquots of plasma and urine were pipetted into Bray's solution, radioactivity was determined in liquid scintillation counting equipment, and PAH extraction and renal plasma flow for the left kidney were calculated as previously described (7). Purified rat renin substrate was prepared as previously described (8). Rat plasma was incubated with rat renin substrate at 37–38° in a shaking water bath for 4 hr; at the end of incubation, the tubes were placed in boiling water for 10 min, then placed on ice while 0.1 ml of 0.88 *N* NaOH was added. After centrifugation, 0.1-ml aliquots of supernatant were injected into urethane anesthetized rats; the pressor responses were bracketed by re-

TABLE I. Data from First Clearance Period.[a]

| | |
|---|---|
| Weight, g | 351.7 ± 10.3 |
| PAH extraction, % | 83.0 ± 0.98 |
| Renal plasma flow, ml/min/kg body weight | 8.11 ± 0.48 |
| Arterial renin, ng angiotensin/ml/hr | 3.14 ± 0.23 |
| Renal venous–arterial renin, ng angiotensin/ml/hr | 1.38 ± 0.25 |
| Renin release rate, ng angiotensin/min/kg/hr | 25.25 ± 14.93 |

[a] Pooled data from 38 rats, mean ± SEM. All parameters except weight and arterial renin are for the left kidney. Renin release rate is the average of values for each rat, not the product of the average RPF and the average RV-A renin.

sponses to 0.1 ml injections of isotonic saline and/or known concentrations of $Val_5$ angiotensin II amide in isotonic saline. Arterial and renal venous renin activities were calculated in ng angiotensin/ml/hr of incubation. The method used for measuring rat plasma renin activity has been validated and used in several previous studies (6, 8–10, 13). In brief, the substrate is essentially free of angiotensinase activity; plasma angiotensinase is inhibited before the incubation by the acidification procedure, during the incubation by EDTA added to the substrate, after the incubation by heating the samples at 100°. Substrate is in excess during the incubation; angiotensin is formed at a constant rate for more than 4 hours of incubation, even when renin concentrations are much higher than those reported here. Renin release rate from the left kidney was calculated as the product of renal plasma flow (RPF) and renal venous minus arterial plasma renin activity (RV—A renin), and is expressed in ng angiotensin/min/hr of incubation/kg body weight. As appropriate, the unpaired and paired $t$ tests were used for statistics.

*Results.* The data from the first clearance periods of 38 rats were pooled and are presented in Table I. PAH extraction was almost identical to previously published values for rats, as was the average renal plasma flow (7). The high extraction indicates that the renal vein catheter was sampling only, or primarily, renal venous blood, uncontaminated with vena caval blood. Arterial plasma renin activity averaged a bit lower than previously published values (11, 12). As expected, renal venous renin activity was higher than arterial.

In 16 rats, two consecutive clearances were performed, during which the rate of intravenous infusion remained unchanged at 0.05 ml/min. Table II contains these results. Using the paired $t$ test, it was determined that none of the measured parameters changed significantly between periods 1 and 2; the blood withdrawn for the first determinations did not increase arterial renin (A renin), renal venous minus arterial renin (RV—A renin), or renin release rate (RPF)(RV—A renin).

Table III presents the effects of saline diuresis. The paired $t$ test was used to determine the significance of the differences in observed means. Saline diuresis reduced A renin and rate of renin release from the kidney.

*Discussion.* The arterial renin activity reported here is slightly lower than previously

TABLE II. Comparison of Consecutive Clearance Periods.[a]

| | Period 1 | Period 2 | |
|---|---|---|---|
| PAH extraction, % | 85.7 ± 1.1 | 83.8 ± 1.3 | (16) |
| Renal plasma flow, ml/min/kg body weight | 9.02 ± 0.8 | 8.66 ± 0.8 | (15) |
| Arterial renin, ng angiotensin/ml/hr | 3.58 ± 0.44 | 2.93 ± 0.39 | (16) |
| Renal venous–arterial renin, ng angiotensin/ml/hr | 1.30 ± 0.52 | 1.26 ± 0.22 | (16) |
| Renin release rate, ng angiotensin/min/kg/hr | 11.12 ± 3.66 | 13.81 ± 4.04 | (14) |

[a] All parameters except weight and arterial renin are for the left kidney. Renin release rate is the average of values for each rat, not the product of the average RPF and the average RV-A renin. Number of paired observations in parentheses. Differences were not statistically significant, $p > 0.05$.

TABLE III. Effects of Saline Diuresis.[a]

| | Period 1 | Period 2 |
|---|---|---|
| PAH extraction, % | 77.8 ± 3.1 | 74.5 ± 3.6 (6) |
| Renal plasma flow, ml/min/kg body weight | 6.6 ± 0.9 | 8.74 ± 0.79 (6) |
| Arterial renin, ng angiotensin/ml/hr | 3.35 ± 0.49 | 1.27 ± 0.19 (6)[b] |
| Renal venous–arterial renin, ng angiotensin/ml/hr | 1.75 ± 0.98 | 0.14 ± 0.09 (6) |
| Renin release rate, ng angiotensin/min/kg/hr | 15.87 ± 7.24 | 1.90 ± 1.38 (6)[c] |

[a] All parameters except weight and arterial renin are for the left kidney. Renin release rate is the average of values for each rat, not the product of the average RPF and the average RV-A renin. Number of paired observations in parentheses.

[b] Diuretic period less than Period 1, $p = 0.004$.

[c] Diuretic period less than Period 1, $p < 0.05$.

reported values for pentobarbital anesthetized rats. Values from 10–14 ng angiotensin/ml/hr of incubation have been reported (11, 12). Differences in substrate preparation, or possibly in experimental condition of the rats, might explain this observation. In the present study, by the time blood was withdrawn for the first renin determinations, about 4 ml of isotonic NaCl had been infused into the rats. Possibly this procedure tended to keep plasma renin activity somewhat lower than in rats whose fluid losses during the experiments were unreplaced.

It has been repeatedly shown that A renin in rats increases as a consequence of hemorrhage (6, 9, 10, 13). As was shown in Table II, the blood loss during the first clearance period was not an adequate stimulus to increase renin release. Because this was so, there was justification in using each rat as his own control when studying the effects of saline diuresis on renin.

Horky *et al.* (12) injected 3 ml of saline into pentobarbital anesthetized rats, and at some later time, measured A and RV renins. They reported that saline decreased neither variable, nor RV—A renin, as compared to a control group of animals. In the present study, renin release was clearly decreased by inducing saline diuresis. A plausible explanation for this apparent divergence of results is that, in the present study, a total of nearly 20 ml per rat of isotonic saline was infused before the second renin determination was made, as compared to the 3 ml saline given to rats by Horkey *et al.* (12). It would be anticipated that saline diuresis would decrease renin release rate, since it does so in dogs (4, 5).

*Summary.* Renin release rate (RPF) (RV—A renin) was measured in pentobarbital anesthetized rats. Plasma renin activities were determined by incubating plasma with purified rat renin substrate and measuring the angiotensin generated during the incubation by bioassay. It was found that the determinations could be made twice in an animal without measurable stimulation of renin release due to blood loss. Saline diuresis was found to inhibit renin release rate, and diminish arterial renin activity in rats.

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