Potassium control of extrarenal renin secretion in transgenic (mRen-2)27 and normal rats

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Rong, Pei, Jennifer L. Wilkinson-Berka, and Sanford L. Skinner. Potassium control of extrarenal renin secretion in transgenic (mRen-2)27 and normal rats. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E631–E638, 1999.—Plasma active renin and prorenin were followed for 12 h after bilateral, unilateral, and sham nephrectomy (BNx, UNx, and SNx) in anesthetized transgenic (mRen-2)27 rats to compare them with Sprague-Dawley and spontaneously hypertensive rats (SDR and SHR). In Ren-2 rats, active renin and prorenin increased with plasma potassium post-BNx and were augmented by potassium infusion. The increase in prorenin but not active renin was abolished by bilateral adrenalectomy (BADRx). However, this did not reduce prorenin below normal, indicating that the high plasma prorenin Ren-2 phenotype is not only of adrenal origin. SNx and UNx also raised plasma active renin and prorenin in Ren-2 rats, with positive correlations to plasma potassium. In SDR and SHR, active renin fell below prorenin post-BNx, and adrenal ablation and potassium loading (in SDR) modified the decreasing active renin profile consistently with low levels of regulated extrarenal secretion. In Ren-2 rats, adrenal but not extra-adrenal prorenin secretion is potassium sensitive and stress related. The unidentified source of active renin in BNx+BADRx Ren-2 rats is also potassium and stress related.

Tissue renin; prorenin; adrenal renin; hypertension; spontaneously hypertensive rat

APART FROM RELEASE of the salivary gland renin in male outbred mice (21) and possibly from the uterus of pregnant rabbits (11, 13) and human ovary (12), enzymatically active tissue renin is not generally acknowledged to have other than a local paracrine role (18, 28). This is despite the fact that anephric humans on long-term dialysis retain ~50% of normal plasma prorenin with active renin and angiotensin (ANG) II variably reported between 5 and 100% of normal (7, 16, 20).

Tissues and cells have been surveyed for renin message and protein, and it is clear that, apart from the kidneys and central nervous system, the adrenal, gonads, uterus, and retina are among the sites with the potential to secrete renin in normal humans and rodents (2, 9, 26). In addition, vascular wall injury induces renin expression in smooth muscle (14), which has strengthened the concept of tissue-based paracrine renin-angiotensin systems (RAS), supporting, for example, intimal hyperplasia in vascular repair, steroid synthesis in adrenals and gonads (5, 23, 30), and cyclical angiogenesis and possibly apoptosis in gonads (8, 12).

Studying renin secretion in vivo from extrarenal sites has been difficult because no experimental animal exhibits the high prorenin phenotype of the normal human, and the initial process of binephrectomy (BNx) to remove renal renin secretion results in very low levels of renin protein in rat plasma. The development of the transgenic Ren-2 rat (17) has assisted the identification of cells throughout the body of even the normal rat that have the potential to synthesize renin (2, 24, 32), and this model might also be useful for the study of normal secretion from these sites.

The Ren-2 rat is a gene dose transgenic strain in which the mouse Ren-2 gene is expressed in parallel with the normal rat structural renin gene, albeit at a higher level (1, 32). Evolutionary alteration in the regulatory elements of the Ren-2 gene has resulted in its enhanced expression in specific tissues of the mouse but does not lead to its random expression as a transgene in rat cells (32). Thus it is expressed in rat kidney, adrenal, and ovary, but in contradistinction to the mouse, Ren-2 is not expressed in the rat salivary glands (25, 32), apparently because the rat lacks the androgen-stimulated transacting factors present in the glands of outbred strains of Mus musculus.

For reasons that have not been clarified, BNx in the Ren-2 rat leads to increasing levels of plasma active renin and prorenin, and biadrenalectomy (BADRx) apparently fails to abolish this response (27). This indicates that tissues apart from adrenal are capable of processing and secreting renin in this transgenic strain, and the possibility exists that this also occurs in normal animals and humans at much lower levels of activity. We have therefore compared the post-BNx profiles of plasma active renin and prorenin in Ren-2 with Sprague-Dawley rats (SDR) and spontaneously hypertensive rats (SHR). To achieve this, it was necessary to improve the sensitivity of plasma renin assays to quantify the low post-BNx levels in normal SDR and SHR. The relationship of post-BNx plasma renin to plasma and dietary potassium was of particular interest, because adrenal renin is known to be stimulated by increased potassium both in vitro and in vivo (10, 30).

METHODS

Rat colonies. Homozygous (HMZ) Ren-2 rats were F1 to F32 inbred progenies of breeding pairs obtained from the Max-Dulbrück Centre, Berlin-Buch, and established in Melbourne in 1993 (see Acknowledgments). All Ren-2 breeding pairs and weanlings received a pellet diet containing 0.76% potassium and 0.25% sodium (GR2 pellets, Clark King, Melbourne, Australia) and drank tap water containing an angiotensin-converting enzyme inhibitor (ACEI, lisinopril; 10 mg/ml, Zeneca, UK; see Acknowledgments) to control hypertension...
and maintain health (17). The approximate daily dose of lisinopril was 0.1 mg·100 g body wt$^{-1}$·day$^{-1}$ calculated from fluid intake. Unless otherwise stated, Ren-2 rats were 12- to 15-wk-old females from which lisinopril was removed 3 wk before experimentation. Rats not receiving lisinopril from weaning displayed conscious systolic blood pressures of 190–250 mmHg after 6 wk, but with lisinopril this was controlled at 130–150 mmHg (tail-cuff systolic pressure; model PE-3500, Narco Bio-Systems, Houston, TX). The 3-wk lisinopril removal period resulted in only 30 to 60 mmHg rises in pressure, but plasma active renin levels normalized, having trebled with lisinopril compared with untreated controls. In contrast, irrespective of lisinopril treatment and the level of blood pressure, plasma prorenin remained relatively constant and not different from untreated Ren-2 controls throughout the entire 15- to 18-wk period. SDR and SHR were from colonies maintained within animal facilities of the Faculty of Medicine, The University of Melbourne, and were fed a pellet diet containing only 0.0002% potassium and 0.4–0.6% sodium (Norco, Lismore, Australia). Rats were housed at 19–21°C with a 12:12-h light-dark cycle. Experiments were approved by the Animal Experimentation Ethics Committee of The University of Melbourne.

BNx was performed after BADRx. Surgical anesthesia was induced with sodium pentobarbital (60 mg/kg ip). Kidneys were exposed through flank incisions, the renal pedicles were ligated, and the kidneys were removed with a minimum of handling. The wounds were closed, and the animals were maintained under light stable anesthesia for up to 12 h (sodium pentobarbital 20 mg/kg 3–5 hourly). After an initial heparin intravenous dose of 80 units, anticoagulation was maintained with 4 units every 2 h. Blood was sampled at intervals from anesthetized rats via an indwelling carotid artery catheter from which pressure was at other times recorded continuously (Matlab, Analog Digital Instruments, Sydney, Australia). Urine was collected over 24 h from a tail vein. Without DEX, angiotensinogen increased to 200%, and systolic BP fell to 91±6 mmHg after 6 wk with lisinopril this was controlled at 130–150 mmHg (tail-cuff systolic pressure; model PE-3500, Narco Bio-Systems, Houston, TX). The 3-wk lisinopril removal period resulted in only 30 to 60 mmHg rises in pressure, but plasma active renin levels normalized, having trebled with lisinopril compared with untreated controls. In contrast, irrespective of lisinopril treatment and the level of blood pressure, plasma prorenin remained relatively constant and not different from untreated Ren-2 controls throughout the entire 15- to 18-wk period. SDR and SHR were from colonies maintained within animal facilities of the Faculty of Medicine, The University of Melbourne, and were fed a pellet diet containing only 0.0002% potassium and 0.4–0.6% sodium (Norco, Lismore, Australia). Rats were housed at 19–21°C with a 12:12-h light-dark cycle. Experiments were approved by the Animal Experimentation Ethics Committee of The University of Melbourne.

To increase the load, a freshly made mush diet containing 1% potassium was fed to SDR for 1 wk before BNx. The objective of this protocol was to enhance the synthesis and secretion of renin in those tissues in which feedback control by ANG II is present. This would include the kidney and adrenal and possibly other tissues such that, after BNx, extrarenal renin secretion might become more obvious. Enalapril was offered in the drinking water to six female SDR [body wt 291±10 g, systolic blood pressure (BP) 106±3.8 mmHg] for 7 days in an individual dose of 30 mg·kg$^{-1}$·day$^{-1}$, and systolic BP fell to 91±3.6 mmHg (P < 0.05, paired t-test). BNx was then performed in one group, and blood samples were collected at intervals under light anesthesia for 12 h. In a second group, rats were allowed to regain consciousness after BNx, and blood samples were collected at 24 and 48 h from a tail vein.
Statistical analysis. Experiments were planned with ≥5 rats to a group. Quantitative values are expressed as means ± SE, and statistical significance was tested using one- or two-way ANOVA (Minitab 10.51) followed by the Newman-Keuls test. The Kruskal-Wallis test was used for nonparametric data.

RESULTS

Profiles of plasma RAS components after BNx ± BADRx in female HMZ Ren-2 rats. Figure 1 presents the increasing levels of active renin, prorenin, and angiotensinogen (aogen) during 12 h under light anesthesia after BNx. The data demonstrate a higher level of prorenin than active renin under conscious preoperative conditions (pro 4.46 ± 1.21 mGU/ml; active 0.145 ± 0.015 mGU/ml; active-to-pro ratio 5.5 ± 2.4%) and a greater absolute rise in prorenin after BNx. The rise in prorenin was virtually abolished by BADRx while leaving active renin unaffected. The post BNx+BADRx prorenin level was, however, not reduced to zero but remained relatively constant and slightly above the normal conscious level P < 0.05 from 4 h. The dose of DEX given after BNx + BADRx successfully resulted in aogen increases equivalent to BNx alone (Fig. 1). A group of male HMZ Ren-2 rats (not shown) was subjected to the same BNx protocol, and identical quantitative renin values both pre-BNx (n = 12) and post-BNx (n = 6) were observed as for females. The other six males were allowed to recover immediately after BNx, and blood was collected 24 h later with animals under anesthesia. The values for active renin and prorenin were both significantly lower at 24 h than at 12 h (active renin, 0.49 ± 0.11 decreased from 1.98 ± 0.36 mGU/ml, P < 0.005; prorenin, 33.6 ± 8.9 decreased from 76.1 ± 11.4 mGU/ml P < 0.02, one-way ANOVA).

Profiles of RAS components after UNx (males) and SNx (females) in HMZ Ren-2 rats. Figure 2 shows that both UNx and SNx were also associated with significant increases in plasma active renin and prorenin. With UNx the profiles for prorenin and aogen were essentially similar to BNx, with active renin slightly lower. With SNx there was no change in aogen, but there were small increases in active renin and prorenin.

Ratios of active renin to prorenin with BNx, BNx+BADRx, UNx, and SNx. Table 1 displays the ratios of active renin to prorenin over the 12-h experimental period. In anesthetized operated female Ren-2 rats, the initial ratio was between 2 and 3%, and this reached 5–6% between 8 and 10 h post-BNx, although this small increase was not statistically significant. The ratios in conscious males, before and after BNx, were similar to those in females (not shown). In contrast, because BADRx inhibited the post-BNx increase in prorenin, the ratio reached 40%, whereas for the UNx and SNx procedures the ratio fell to ~1%.

Relationship between potassium and RAS components after BNx with and without BADRx in female HMZ Ren-2 rats. Even without potassium infusion, plasma potassium increased progressively during each 12-h protocol, and there were significant regressions for plasma active renin and prorenin on plasma potassium after both SNx and BNx in Ren-2 rats (Fig. 3). The 6-h infusion of potassium commencing at BNx increased both active renin and prorenin significantly at 4, 8, 10, and 12 h post-BNx and markedly extended the relationship of renin and prorenin to plasma potassium (Fig. 3). Plots of plasma potassium against the corresponding active renin or prorenin estimations for BNx, BNx + potassium, and SNx protocols yielded high positive correlations (Table 2). When BNx was combined with BADRx, the active renin-to-potassium correlation re-
mained unchanged, but for prorenin the relationship was abolished (Table 2). For each protocol, the slopes of the regressions ($K$) of the respective renin form on potassium were similar except for active renin after SNx, which was significantly lower, and for prorenin with BADRx, which was zero (Table 2).

Profiles of plasma active renin and prorenin after BNx in female SDR and SHR. BNx in SDR and SHR resulted as expected in rapid declines in active renin and prorenin (Fig. 4). In both rat strains, prorenin was initially less than active renin, but at 2–4 h this reversed; active renin fell more rapidly, and prorenin remained significantly higher for the subsequent 10- to 12-h period. Despite the fact that the initial resting levels of renin and prorenin were higher in SDR than in SHR, post-BNx prorenin in SHR did not decrease

### Table 1. Ratios expressed as percentages of active renin to prorenin in plasma in Ren-2 rats after surgical procedures

<table>
<thead>
<tr>
<th>Time, h</th>
<th>BNx</th>
<th>BNx + BADRx</th>
<th>UNx</th>
<th>SNx</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.6 ± 0.3</td>
<td>4.1 ± 1.1</td>
<td>3.6 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>0.5</td>
<td>2.6 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>3.6 ± 0.8</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>4.7 ± 0.9</td>
<td>13.3 ± 1.1†</td>
<td>3.7 ± 0.3</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>5.8 ± 1.3</td>
<td>20.1 ± 3.2†</td>
<td>1.3 ± 0.2†</td>
<td>1.4 ± 0.4‡</td>
</tr>
<tr>
<td>10</td>
<td>5.4 ± 1.8</td>
<td>31.9 ± 4.3†</td>
<td>1.1 ± 0.1†</td>
<td>1.6 ± 0.4*</td>
</tr>
<tr>
<td>12</td>
<td>3.5 ± 0.8</td>
<td>40.7 ± 10.5†</td>
<td>0.8 ± 0.1†</td>
<td>1.5 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE of ratios shown in % derived from data shown also in Figs. 1 & 2. BNx, bilateral nephrectomy; BNx + BADRx, BNx + bilateral adrenalectomy; UNx, unilateral nephrectomy; SNx, sham nephrectomy. Significance levels refer to changes from time 0. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ by one-way ANOVA with Newman-Keuls test.

Fig. 3. Regressions of plasma active renin (A) and prorenin (B) on plasma potassium in sham nephrectomy (SNx, ○, $n = 6$) and male uninephrectomy (UNx, ■, $n = 5$) procedures in anesthetized HMZ female Ren-2 rats. For each group (active renin, A; prorenin, B; angiotensinogen, C), statistical comparisons are with initial anesthetized level. Changes with time should not be compared between groups because of the different genders. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by one-way ANOVA.
further beyond 4 h and became significantly higher than SDR prorenin at 10 h (SHR, 29.6 ± 4.8 vs. SDR, 11.6 ± 0.1 µGU/ml, *P*, <0.05 by two-way ANOVA with Neuman-Keuls test). The profiles of aogen in SDR and SHR were not different, and both reached the same level as Ren-2 rats by 10–12 h.

Profiles of plasma active renin and prorenin after BNx combined with either BADRx or a high-potassium diet in female SDR. In further groups, a slightly lower decay profile of active renin, but not prorenin, was associated with removal of the adrenals (with DEX replacement) after BNx in SDR (Fig. 5). The resting level of active renin was not affected by the 1% potassium diet, but the post-BNx active renin profile was slightly elevated (Fig. 5). Prorenin measured only at 8–12 h post-BNx was not affected by the potassium diet.

Effect of prior treatment with enalapril on the post-BNx profiles of active renin and prorenin in SDR. ACEI treatment for 7 days raised plasma active renin 15-fold and prorenin 31-fold. At 30 min after BNx, active renin decreased by 24% and prorenin by 29%. These can be compared with 64 and 48% decreases, respectively, in untreated SDR in Fig. 4. At 12 and 24 h, the renin levels in enalapril-treated rats were still well above even the normal levels in untreated intact rats, but by 48 h active renin (prorenin not assayed) had decreased to the level expected at 12 h post-BNx in untreated rats.

DISCUSSION

The present experiments shed new light on the mechanism of increasing plasma active renin and prorenin after BNx in the Ren-2 rat. Over a 12-h post-BNx period, progressive increases in active renin and prorenin related closely to increasing plasma potassium levels and were augmented further by potassium infusion. For prorenin, the rise but not the normal background level was abolished by BADRx. This confirms that the post-BNx increase in plasma prorenin is of adrenal origin (27) but also indicates that the normal high plasma prorenin phenotype of the Ren-2 rat is not due to adrenal secretion and comes from sources that are not greatly influenced by potassium or surgical stress. The BNx+BADRx experiment reveals for the first time that increasing active renin is from an extra-adrenal source, from which secretion is nevertheless potassium related. Even SNx and UNx procedures were associated with parallel increases in plasma potassium and prorenin, indicating that, unrelated to the anephric state, surgical stress has an influence on adrenal prorenin secretion in the Ren-2 rat. But the same stress had less effect on active renin secretion from the unidentified sources, as evidenced by the UNx and SNx procedures.

We considered the possibility that some of the post-BNx increase in active renin might be artifactual, resulting from a minor degree of intrinsic enzymatic...
activity of prorenin or from its activation in vitro. This at first seemed likely because of the relatively constant active-to-prorenin ratio during the post-BNx increases in plasma renin, but the specific effect of BADRx on prorenin denied this possibility. In addition, we have not seen any activation of prorenin in Ren-2 rat plasma during many months of storage, under the present conditions or with thawing and freezing. The possibility that the increasing plasma aogen post-BNx might itself lead to an overestimation of renin concentration was excluded, because the contribution of the endogenous aogen was never more than 10% of the total in the incubate. The BADRx result, and the fact that both UNx and SNx produced increases in prorenin and that potassium infusion accentuated the rises in active renin and prorenin, exclude the possibility that the increases resulted from renin protein accumulation due to decreased clearances in the anephric state or to inadvertent estimation errors. Increased secretion of each renin form is the logical conclusion consistent with the findings.

Concerning the unidentified extrarenal source secreting active renin in the BNx Ren-2 rat, several conclusions can be drawn. As for kidney, the tissue is able to process renin to its fully mature form and, apparently, to secrete it apart from any prorenin secretion. This indicates the intracellular presence of processing enzymes and implies physiological significance. Up to 12 h post-BNx, secretion increases in the same linear manner as for adrenal prorenin and is potassium related. Although SNx had only a small effect on active renin secretion, the source and stimulus would nevertheless appear to be stress related, because plasma potassium increased even with this minimal procedure, and the relevant relationships were highly significant.

Having quantified and characterized these stress-related increases in extrarenal renin secretion in Ren-2 rats, we sought to answer the more general biological question of whether extrarenal RAS activation and secretion might also be relevant to pathophysiological processes involving the renin structural gene in normal rats and, ultimately, humans. The adrenal is an acknowledged site of tissue renin synthesis and stores both active renin and prorenin in normal rodents and humans. It responds to BNx with marked increases in content of both renin forms, and potassium is a potent stimulus to secretion of both active renin and prorenin from normal and Ren-2 rat adrenal cortical cells (4, 10, 30). In addition, adrenal steroid cells of both normal and Ren-2 rats display the same unique intracellular mitochondrial and cytoplasmic distribution of active renin and prorenin (22, 24), although in Ren-2 rats, as in mice, both zona glomerulosa and fasciculata express renin, and the content is higher in Ren-2 rats (1, 17, 31). It is perhaps surprising, therefore, that adrenal renin secretion into plasma has as yet not been demonstrated in normal animals in vivo. This situation has led us to conjecture that previous failures may be due to the limits of renin quantification and experimental design.

In pursuit of this concept, we repeated the BNx+BADRx ablation experiment combined with a potassium loading in SDR and found some, but not strong, evidence of adrenal renin secretion. In planning this experiment, a 12-h observation period was considered as most appropriate, because longer periods might deplete the renin content of extrarenal tissue and reduce its secretion. This view was supported by a 24-h study in BNx male Ren-2 rats in which the levels of renin and prorenin fell from the higher 12-h level. On the other hand, previous workers addressing this question have assumed that if post-BNx renin levels are lower at, say, 24 or 48 h than earlier, it simply indicates continuing clearance of previously secreted renal renin from tissue spaces or uptake sites (6). Our findings in SDR of a decreased profile of active renin with BADRx over the 12 h after BNx and an increased profile with a higher potassium intake (without a rise in the pre-BNx resting level of renin) suggest low secretion from the adrenal for 12 h. Clearly this is only suggestive evidence, but it has led us to commence arteriovenous difference studies across the adrenals and other tissues.

Fig. 5. Effect of BADRx or dietary potassium supplementation on plasma active renin (A) and prorenin (B) after BNx in SDR (logarithmic scales). BNx control for BADRx (○, n = 5); BNx+BADRx (●, n = 6); BNx on low K+ diet (□, n = 6); BNx after augmentation of K+ in diet (■, n = 6). For active renin, BADRx decreased profile (● vs. ○, **P < 0.01) and potassium increased profile (□ vs. ○, *P < 0.05), but there were no significant effects on prorenin.
in SDR in which significant but low levels of renin secretion have been recorded (unpublished data). With respect to this question in the present study, it was incorrectly predicted that by raising plasma renin levels with an ACEI before BNx, the impact of stimulated extrarenal renin secretion might become obvious after the rapid clearance of renal renin from the tissue spaces. In the event, the decay curves in enalapril-treated BNx SDR do not allow any such conclusion and simply demonstrate a greatly protracted renin decay profile consistent with clearance of previously secreted renal renin, although not excluding an extrarenal contribution.

We have recently used the Ren-2 rat to explore the role of the tissue RAS in the pathogenesis of diabetic nephropathy and have produced the first rodent model of severe glomerular and tubulo-interstitial disease similar to end-stage human diabetic renal failure (15). The nephropathy is apparently unrelated to the hyperglycemia and hyperlipidemia seen in diabetic rats (16) and may be more similar to end-stage human diabetic renal failure (15). The pathophysiology of prorenin. In: The Renin-Angiotensin System, edited by J. I. Robertson and M. G. Nicholls. London: Gower Medical Publishing, 1993.

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