CARDIOVASCULAR EFFECTS OF PRORENIN BLOCKADE IN
GENETICALLY HYPERTENSIVE RATS (SHR) ON NORMAL AND HIGH
SALT DIET

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Running head: Prorenin and the heart

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Abstract

Recent reports have demonstrated a potential role of tissue prorenin in the pathogenesis of cardiovascular and renal damage. This study was designed to examine the role of prorenin in the pathogenesis of target organ damage in spontaneously hypertensive rats, the best naturally occurring experimental model of essential hypertension. To this end, we studied 20-week-old male SHR receiving a normal diet and 8-week old male SHR given food with 8% NaCl. One-half the rats in each group were given prorenin inhibitor (PRAM 1, 0.1 mg/kg/day) via osmotic mini-pumps; the other half served as controls. Arterial pressure, left ventricular function, cardiovascular mass indices, cardiac fibrosis, and renal function were examined at the end of the experiment. Arterial pressure was unaffected by PRAM 1 in rats on either regular or salt-excess diets. In those rats receiving a normal diet, blockade of prorenin activation consistently reduced left ventricular mass, but affected no other variable. Salt-loaded rats given PRAM1 for 8 weeks demonstrated: (i) reduced serum creatinine level, (ii) decreased left ventricular mass, (iii) improved left ventricular function, and (iv) reduced left ventricular fibrosis. These data demonstrated that blockade of nonproteolytic activation of prorenin exerted significant cardiovascular and renal benefit in SHR with cardiovascular damage produced by salt excess and suggested that activation of cardiovascular or renal prorenin may be a major mechanism that mediates cardiac and renal damage in this form of accelerated hypertension.

**Key words:** Renin, Prorenin, Prorenin inhibitor, Proteinuria, Left ventricular function, Myocardial fibrosis
Renin has long been suggested to exert its action independent of the classical circulating renin-angiotensin system (RAS), whereby formation of angiotensin II in circulating blood is a crucial event in its mechanism of adverse actions (5). It is now well-established that angiotensin II is also formed locally, in various tissues, thereby exerting its actions independent of circulating RAS (2,13,18). Furthermore, three distinct renin receptors on various cells are known to exist (15). In addition to renin, all three receptors also bind prorenin, the inactive precursor of renin that is enzymatically transformed into the active form, “mature” renin, by cleavage of a 43-amino acid segment from the amino terminus, a process exclusively confined to the cells of juxtaglomerular apparatus (15). Prorenin is not only formed in kidney, but also by other tissues, and its plasma concentration in normal subjects is about ten times higher than renin (16). This high plasma prorenin level suggests that it may have physiological effects, but its functional significance has yet to be determined. It has been suggested that plasma prorenin may be used as a marker of microvascular complications of diabetes, although not the most reliable one (11). The newly described prorenin receptors provide a possible mechanism for the functional role of prorenin. When the “handle region” of prorenin is bound to a putative specific receptor, it changes its conformation to become enzymatically active, comparable to the activity of renin (7,15). In addition, interaction of both renin and prorenin with the receptors triggers intracellular signaling that activates protein kinases and may ultimately lead to fibrosis independent of circulating RAS (7,15). A recent publication on kinase activation in monocytes indicates that prorenin and renin induced activation of kinase are independent of angiotensin II (3).
The synthetic handle region peptide that binds to the prorenin receptor, thereby competitively inhibiting prorenin binding and consequently preventing angiotensin generation and RAS-independent intracellular signaling by prorenin has been synthetized (7). It has also been reported that this synthetic peptide binds to (pro)renin receptor and inhibits prorenin activation (17). This peptide has also been used in several recent studies that demonstrate a potential role of prorenin in the pathogenesis of diabetes mellitus and hypertension (6,8,9). Thus, in rats with streptozotocin-induced diabetes, administration of the handle region peptide prevented development of nephropathy and proteinuria (6). Similarly, in diabetic mice with angiotensin II type 1a receptor deficiency, treatment with the handle region peptide prevented development of glomerulosclerosis by abolishing the increased mitogen-activated protein kinase activation (9). Further, in the salt-loaded stroke-prone spontaneously hypertensive rat, eight-week treatment with the prorenin inhibitor decreased cardiac angiotensin II levels and attenuated development and progression of cardiac fibrosis without affecting elements of circulating RAS or arterial pressure (8). This study was designed to examine the role of prorenin in the pathogenesis of cardiovascular damage in spontaneously hypertensive rats, the best existing naturally occurring experimental model of essential hypertension.

**Materials and Methods**

**Experimental Animals.** Male SHR, purchased from Harlan Laboratories (Indianapolis, IN), were maintained in a temperature and humidity-controlled room with a 12 hours light/dark cycle. All rats were handled in accordance with National Institute of Health guidelines; and our Institutional Animal Care and Use Committee approved the study protocol in advance. Male, 20-week old SHR rats were divided into two groups (12
rats in each) which were implanted subcutaneously with osmotic mini-pumps (Alzet, Model 2ML4 for 28 days) containing either the handle peptide, PRAM1 (Figure 5) (concentration was adjusted to deliver PRAM in a dose of 0.1 mg/kg/day) or an inert vehicle (distilled water). The peptide, custom synthesized by Biospace, Inc. or Washington Biotechnology, Inc. Gaithersburg, MD was >99% pure with the correct MW by mass spectrometry. Systemic and regional (including coronary) hemodynamics, left ventricular function (using catheter-tip transducer), and cardiovascular mass indices were determined at the fourth week of treatment. The study was repeated in an experimental model with cardiovascular and renal damage aggravated by salt overload (12,24). To this end, male, 8 week old SHR, were given rodent chow containing 8% NaCl and were divided into two groups: one group (n=12) was implanted with osmotic mini-pumps (Alzet, Model 2ML4 for 28 days) containing prorenin inhibitor (PRAM 1, 0.1 mg/kg/day) and the other (n=13) received pumps with inert vehicle. Arterial pressure, left ventricular (LV) function, cardiovascular mass indices, degree of cardiac fibrosis and renal functional indices were examined at the end of the 8-week course of the experiment (mini-pumps were replaced after 4 weeks). The concentration of PRAM1 in the mini-pumps after 4 weeks was determined by HPLC and found to be 0.44 ug/ml, 88.2% of the original solution. In addition, no degradation products of the peptide were observed. All rats were permitted free access to their respective chow and tap water.

**Twenty Four-Hour Urinary Measurements.** During the last week of treatment, all rats were placed in individual metabolic cages for three consecutive days. Urine was collected during the second and third day; urinary output was measured and 24-hr urinary protein (Lowry method) excretions were determined.
Systemic and Regional Hemodynamics and Left Ventricular Function. At the end of the study, all rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and the right carotid artery was cannulated with a transducer-tip catheter (Micro-Tip 3F, Millar Instruments) that was advanced into the left ventricle (LV). A second catheter (PE-50) was placed into femoral artery. Both catheters were connected to a multi-channel recorder (Grass Instrument) interfaced to an IBM computer with digital data acquisition system (EMKA Technologies Inc) (24). Arterial pressure was measured via femoral artery catheter and indexes of left ventricular function, including LV end-diastolic pressure, diastolic time constant (tau) and maximal rates of pressure rise and decline (dP/dT\text{max} and dP/dT\text{min}) were determined from LV pressure tracing. After these measurements were obtained, the Millar catheter was withdrawn and a jugular vein and left ventricle were cannulated with polyethylene catheters (PE-50) for determination of systemic, coronary and renal hemodynamics (using radio-labeled microspheres) as described previously (21,22). After the regional hemodynamic study, rats were killed with an overdose of pentobarbital and their heart, aorta, and kidneys were removed and weighed. As an estimate of ventricular collagen content, hydroxyproline concentration in the LV samples was determined and expressed as mg/g of dry weight (21, 22).

Statistical Analysis. All values are expressed as the mean ±1SEM. Data were analyzed by unpaired t-test (1). A value of p<0.05 was considered to be of statistical significance.

Results

Administration of the prorenin inhibitor consistently decreased LV mass in SHR rats on the normal salt diet, but affected no other examined variables including arterial
pressure, cardiac output, total peripheral resistance, LV function, and blood flow and vascular resistance of brain, kidney, and heart (Table 1).

In salt-loaded SHR rats given vehicle, 3 rats developed heart failure (labored breathing, pulmonary edema, and increased LV end-diastolic pressure) and one rat developed signs of stroke. In salt-loaded rats given PRAM 1, only one rat developed heart failure. The data obtained from these rats were excluded.

Body weight was lower in salt-loaded SHR rats given vehicle than in PRAM1 treated rats (278 ± 10 g vs. 310 ± 8 g; p<0.05). No differences in arterial pressure and heart rate were observed between the two groups of salt-loaded rats (Figure 2). Furthermore, no difference in right ventricular mass index was observed between the groups, but LV mass index, LV hydroxyproline concentration and kidney mass index were lower in the salt-loaded rats given PRAM1 than in these rats given vehicle (Figure 3), indicating reduced LV and renal mass as well as diminished myocardial fibrosis. As compared with controls, salt-overloaded rats given PRAM1 demonstrated (after 8 weeks treatment) improved renal function as indicated by a slight, but not significant decrease in urinary protein excretion and significant decrease in serum creatinine level (Figure 4). When compared with their controls, LV diastolic function was improved in the salt-loaded rats given PRAM1, as indicated by a decreased diastolic time constant and improved maximal rate of pressure decline (-dP/dT) (Figure 5). There was no difference in LV end-diastolic pressure between the groups (Figure 5). Finally, maximal rate of pressure rise, as an index of systolic function, was somewhat increased (but not statistically so) in the salt-loaded PRAM1 rats.
Discussion

These data demonstrated that blockade of nonenzymatic prorenin activation did not affect systemic hemodynamics but consistently reduced LV mass in SHR rats on normal salt diet. These findings are in agreement with previous findings in stroke-prone SHR rats (8). However, we did not demonstrate any effect of prorenin blockade on myocardial collagen content, LV function, and coronary and renal hemodynamics in rats not salt-loaded. This lack of effects does not seem to be a consequence of a relatively short treatment period, since we have shown previously that 3-week treatment of young adult SHR with angiotensin II type 1 receptor antagonists (10) effectively reduced LV mass and fibrosis and improved coronary hemodynamics.

The present results further demonstrated that blockade of prorenin exerted significant beneficial cardiovascular and renal effects in salt loaded SHR. Thus, these rats given prorenin inhibitor demonstrated substantial reductions in LV mass and myocardial fibrosis. Furthermore, our results demonstrated, for the first time, significant improvements in LV and renal functions without changing arterial pressure in salt-loaded SHR given prorenin inhibitor. These findings confirm and further extend the earlier observations that administration of the handle peptide prevented myocardial fibrosis in stroke-prone SHR without affecting arterial pressure and circulating elements of the RAS (8). However, in contrast, the results of a recent study indicate that blockade of (pro)renin receptor does not improve target organ damage in rats with renovascular hypertension (14). The fact that blockade of prorenin exerted significant beneficial cardiovascular effect in salt-loaded SHR in the present study, as well as in salt-loaded stroke-prone rats (8), but not in some other forms of hypertension suggests that contribution of prorenin to
end-organ damage may be limited to acute and more severe forms of hypertension, such as in salt-loaded models (20). In the SHR with a naturally-occurring form of hypertension, cardiac and renal damage progress slower and prorenin inhibition may have limited influence on end-organ damage; however this notion requires further investigation since a number of other possibilities exist. In fact, beneficial effects of (pro)renin receptor blockade have so far been noted only in hypertensive models in which target organ damage was aggravated by dietary salt excess, indicating that pro(renin) receptors may be involved in mediating salt-induced cardiovascular and renal injury.

This study did not focus on the exact mechanism of action of PRAM1. One possibility might be that blockade of nonproteolytic activation of local tissue prorenin occurs in heart and kidney. We have suggested previously that the local cardiac RAS may actually mediate cardiovascular injury in salt-overload (4,23,24), and the present findings are in agreement with that concept. Furthermore, we have also reported that similar to the present data, AT1 receptor blockade attenuated adverse cardiovascular and renal effects of salt excess in SHR without affecting arterial pressure (25). All these findings support the notion that the local RAS (including prorenin) participates in mediating cardiovascular and renal damage in animals given salt-excess.

Finally, it is worth noting that recent discovery of prorenin receptors introduces a new possible mechanism of action for components of RAS. Thus, when bound to receptors, renin and prorenin may trigger intracellular signaling that will eventually result in adverse cardiovascular events independent of the RAS. Furthermore, prorenin bound to its receptor, may become active and mediate the local tissue formation of angiotensin II
with related adverse consequences. Therefore, development of specific prorenin blockers may launch an important new therapeutic avenue, in addition to the already-existing RAS inhibitors.
References


contributes to development of cardiac fibrosis in genetic hypertension.


Myocardial fibrosis, impaired coronary hemodynamics, and biventricular

receptor antagonism attenuates target organ effects of salt excess in SHRs without
**Figure Legends**

**Figure 1.** Systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressure and heart rate (HR) in SHR given salt overload for eight weeks and treated with either prorenin inhibitor (PRAM1) or vehicle. Values are mean ± 1SEM. Nine animals in vehicle group and 11 rats in PRAM1 group.

**Figure 2.** Right (RVMI) and left (LVMI) ventricular mass indexes, left ventricular hydroxyproline (LVHy) in SHR given salt overload for eight weeks and treated with either prorenin inhibitor (PRAM1) or vehicle. Values are mean ± 1SEM. Nine animals in vehicle group and 11 rats in PRAM1 group. *p<0.05

**Figure 3.** Urine volume (UV), urinary sodium excretion (Na), urinary protein excretion and serum creatinine in SHR given salt overload for eight weeks and treated with either prorenin inhibitor (PRAM1) or vehicle. Values are mean ± 1SEM. Nine animals in vehicle group and 11 rats in PRAM1 group.

**Figure 4.** Left ventricular end-diastolic pressure (LVEDP), diastolic time constant (Tau), maximal rates of left ventricular pressure rise (+dP/dTmax) and decline (-dP/dTmax) in SHR given salt overload for eight weeks and treated with either prorenin inhibitor (PRAM1) or vehicle. Values are mean ± 1SEM. Nine animals in vehicle group and 11 rats in PRAM1 group. *p<0.05

**Figure 5.** Amino acid sequence (rat) of segment at amino end of prorenin that is cleaved in proteolytic activation. Nonproteolytic prorenin activation inhibitor is designed from handle region (underlined) (6).
Table 1. Cardiovascular effects of short-term treatment with PRAM1 (4 weeks) in spontaneously hypertensive rats on normal salt diet.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PRAM1</th>
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<tr>
<td><strong>Mass Indexes</strong></td>
<td></td>
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<tr>
<td>Body Weight (g)</td>
<td>424 ± 6</td>
<td>444 ± 8</td>
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<tr>
<td>Left Ventricular Weight Index (mg/g)</td>
<td>2.81 ± 0.04</td>
<td>2.59 ± 0.03*</td>
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<tr>
<td>Right Ventricular Weight Index (mg/g)</td>
<td>0.60 ± 0.02</td>
<td>0.57 ± 0.02</td>
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<td>Aortic Weight Index (mg/mm)</td>
<td>1.46 ± 0.04</td>
<td>1.36 ± 0.04</td>
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<td><strong>Systemic Hemodynamics</strong></td>
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<tr>
<td>Systolic Pressure (mmHg)</td>
<td>253 ± 6</td>
<td>231 ± 11</td>
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<tr>
<td>Diastolic Pressure (mmHg)</td>
<td>183 ± 4</td>
<td>162 ± 9</td>
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<tr>
<td>Mean Pressure (mmHg)</td>
<td>208 ± 7</td>
<td>189 ± 3</td>
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<tr>
<td>Heart Rate (beats/min)</td>
<td>409 ± 7</td>
<td>396 ± 12</td>
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<tr>
<td>Cardiac Index (ml/min/kg)</td>
<td>156 ± 3</td>
<td>164 ± 13</td>
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<tr>
<td>Total Peripheral Resistance (u)</td>
<td>1.34 ± 0.05</td>
<td>1.19 ± 0.03</td>
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<td><strong>Kidneys</strong></td>
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<tr>
<td>Blood Flow ml/min/g</td>
<td>4.59 ± 0.31</td>
<td>5.08 ± 0.27</td>
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<tr>
<td>Vascular Resistance u</td>
<td>47.3 ± 5.8</td>
<td>37.8 ± 2.6</td>
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<td><strong>Brain</strong></td>
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<td>Blood Flow (ml/min/g)</td>
<td>0.832 ± 0.090</td>
<td>0.871 ± 0.043</td>
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<tr>
<td>Vascular Resistance (u)</td>
<td>262 ± 23</td>
<td>237 ± 35</td>
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<tr>
<td><strong>Left Ventricle</strong></td>
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<tr>
<td>Blood Flow (ml/min/g)</td>
<td>4.26 ± 0.18</td>
<td>4.48 ± 0.33</td>
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<tr>
<td>Vascular Resistance (u)</td>
<td>49.4 ± 2.9</td>
<td>43.7 ± 4.5</td>
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<tr>
<td>Minimal Vascular Resistance (u)</td>
<td>16.0 ± 0.5</td>
<td>14.4 ± 0.6</td>
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<td>Flow Reserve (ml/min/g)</td>
<td>2.01 ± 0.42</td>
<td>2.76 ± 0.66</td>
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<tr>
<td><strong>Left Ventricular Function</strong></td>
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<tr>
<td>End-Diastolic Pressure (mmHg)</td>
<td>-1.28 ± 0.89</td>
<td>-2.68 ± 0.86</td>
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<tr>
<td>Maximal Rate of Pressure Rise (mmHg/s)</td>
<td>10842 ± 470</td>
<td>10346 ± 734</td>
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<tr>
<td>Maximal Rate of Pressure Decline (mmHg/s)</td>
<td>-8516 ± 627</td>
<td>-8047 ± 324</td>
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<tr>
<td>Diastolic Time Constant (msec)</td>
<td>11.15 ± 0.35</td>
<td>9.44 ± 0.73</td>
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Values are means ± 1 SEM. *p<0.05. 12 animals in each group
Figure 1.
Figure 2
Figure 3
Figure 4
B ORIGIN OF PRORENNIN INHIBITOR STRUCTURE

43-AA prosegment
Prorenin: LPTDTASFGRILLKKKMPSVREIL-
EERGVDMTRISAEWGEFIKK-Renin

PRAM1: RILLKKMPSV
(Handle region peptide)