The Renin Response to Diuretic Therapy

A Limitation of Antihypertensive Potential

E. DARRACOTT VAUGHAN, JR., ROBERT M. CAREY, MICHAEL J. PEACH, JOHN A. ACKERLY, AND CARLOS R. AYERS

SUMMARY We attempted to determine whether a diuretic-induced increase in renin secretion results in angiotensin II-mediated vasoconstriction which counteracts the antihypertensive action of diuretics. The angiotensin antagonist saralasin was administered to nine normal renin and five low renin essential hypertensives prior to and during stimulation of the renin-angiotensin system by 6 weeks of chronic diuretic therapy alone. Initial responses to saralasin infusion in all subjects on placebo varied, but were pressor overall. Following chronic polythiazide therapy, saralasin induced depressor responses of variable magnitude in seven of nine normal renin subjects. The combination of the diuretic and saralasin normalized blood pressure in four subjects. However, four normal renin subjects maintained a diastolic blood pressure greater than 95 mm Hg despite simultaneous volume depletion and angiotensin blockade. One had a maximum antihypertensive effect with diuretic therapy alone. Pressor responses to saralasin persisted in four of five low renin subjects despite diuretic therapy. In general, the magnitude of the change in plasma renin activity induced by diuretic therapy correlated with the difference in blood pressure responses to saralasin. However, individual blood pressure responses and absolute renin levels after diuretic therapy failed to predict consistently the ensuing responses to saralasin. Hence, in eight subjects (seven normal and one low renin), the compensatory increase in renin secretion in response to diuretic-induced volume depletion limited diuretic antihypertensive efficacy. Yet diuretic-induced angiotensin dependency was not the sole mechanism supporting residual hypertension in all subjects refractory to diuretic therapy.

IONS THE development of chlorothiazide in 1957, diuretic agents have remained the foundation of antihypertensive therapy. However, except for patients with essential hypertension and low plasma renin activity whose blood pressures normalize with diuretics, blood pressures in perhaps 75% of essential hypertensive patients do not normalize with diuretic-induced sodium and volume loss.

A physiological response to diuretic-induced sodium and volume loss is a reactive rise in plasma renin activity which persists throughout therapy and abates upon withdrawal of the drug. However, the total effect of angiotensin II on blood pressure has been difficult to document, because sodium-volume depletion decreases vascular responsiveness to this pressor peptide.

The use of angiotensin II antagonists which compete with angiotensin II at vascular receptors and thereby decrease blood pressure has implicated angiotensin II in blood pressure maintenance. In addition, intensive short-term sodium depletion, which induces renin secretion, has unmasked angiotensin II dependency even in hospitalized patients with initially low plasma renin activity.

Hence, if a diuretic-induced increase in renin secretion results in angiotensin II-induced vasoconstriction, then the administration of an angiotensin II analogue such as [Sar'-Ala']-angiotensin II (saralasin) should result in a depressor response.

The present study was designed to determine whether the reactive increase in renin secretion induced by chronic diuretic therapy in outpatients results in angiotensin II-dependent vasoconstriction which attenuates the antihypertensive efficacy of diuretics. Normal and low renin hypertensive patients were infused with saralasin prior to and during chronic conventional diuretic therapy in order to quantify any change in the angiotensin II contribution to blood pressure maintenance.

Methods

Fourteen patients diagnosed as essential hypertensives by normal renal arteriograms and laboratory tests were selected from the University of Virginia Hypertension Clinic. All antihypertensive medication was stopped and the subjects were placed on a single-blind placebo regimen for 5 weeks. During the 5th week, subjects were placed on 2 g/day sodium diets as outpatients; after 5 days, venous blood samples were taken at noon from upright ambulatory patients to determine plasma renin activity which was indexed against the concurrent 24-hour urinary sodium excretion by the method of Laragh et al. (Figure 1, left panel) shows the renin-sodium index for each subject. The dotted lines define limits, established in our laboratory, of the normal inverse hyperbolic relationship between plasma renin activity and sodium excretion. In addition, all subjects underwent oral furosemide tests (Fig. 1, right panel), as described by Carey et al. In five
identified as low renin essential hypertensives by the renin-sodium index, plasma renin activity did not rise above 1.7 ng/ml per hour in response to furosemide.2

At the end of the 5th week on placebo, the subjects were brought to the Clinical Research Center for saralasin infusion. Antecubital intravenous infusions were started, and supine blood pressures were monitored with an arteriosonde 1216 (Roche Instruments, Inc.) at 2-minute intervals for 30 minutes. Saralasin (0.78 mg/min) was then infused with a Harvard-type pump for 30 minutes. Plasma renin activity was determined from venous blood samples drawn from the contralateral arm 10 minutes prior to and immediately after infusion. After termination of infusion, blood pressures were monitored for an additional 30 minutes. The control pressure is defined as the mean of 10 readings taken every 2 minutes prior to infusion. The effect of saralasin was determined by averaging the 2-minute readings at the midpoint (12 to 22 minutes) of infusion after any immediate transient agonist effect of the analogue had subsided. A change in blood pressure is defined as the difference between these averaged values for the control and experimental periods. The final blood pressure represents a mean of five readings taken 20-30 minutes after termination of infusion.

After saralasin infusion, subjects were placed on polythiazide (Renese) 1 mg three times daily, and followed in the Hypertension Clinic every 2 weeks. After 6 weeks, the protocol for saralasin infusion, blood pressure determinations, and blood sampling for plasma renin activity was repeated.

Written informed consent explaining the experimental design and the experimental use of saralasin was obtained from each subject.

Analytical Methods

After incubation, plasma renin activity was determined by radioimmunoassay of angiotensin I as described by Sealey et al.10 Results are expressed as mean ± standard error. Statistical analysis is by paired or unpaired Student’s t-test, and P values (double-tailed) less than or equal to 0.05 are considered significant. Correlations were evaluated with the Pearson product moment correlation coefficient.

Results

Effect of Polythiazide and Saralasin on Plasma Renin Activity

By both methods of renin classification (Fig. 1), nine subjects were defined as normal renin and five as low renin hypertensives. All exhibited rises in supine plasma renin activity in response to polythiazide therapy (Table 1). Absolute changes in plasma renin activity in response to diuretic therapy were greater in normal than in low renin subjects. After saralasin infusion, renin values fell slightly in normal renin subjects taking placebo but were unchanged in low renin hypertensives. With diuretic therapy, plasma renin activity rose variably in response to saralasin in five of nine normal renin subjects but fell in all low renin subjects (P < 0.05).

Effect of Saralasin on Blood Pressure Prior to and after Polythiazide

Individual subject characteristics and systolic and diastolic blood pressures at each phase of the study are given in Table 2. Individual changes in diastolic blood pressure after saralasin infusion during administration of either placebo or polythiazide are shown in Figure 2. After placebo, blood pressure responses to saralasin were variable but usually pressor in both groups, with a maximum

![Figure 1](image-url)  
**Figure 1** Renin typing of the 14 subjects by sodium-renin index (left panel) and furosemide stimulation (right panel) identifying nine normal renin and five low renin essential hypertensives. The dotted lines define the limits of the normal inverse hyperbolic relationship established in our laboratory between plasma renin activity and sodium excretion in normal subjects.

![Table 1](image-url)  
**Table 1** Supine Plasma Renin Activity before and Immediately after Each Saralasin Infusion

<table>
<thead>
<tr>
<th></th>
<th>Placebo pre-saralasin</th>
<th>Placebo post-saralasin</th>
<th>Diuretic pre-saralasin</th>
<th>Diuretic post-saralasin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal renin</td>
<td>1.07 ± 0.61</td>
<td>29.20 ± 65.80</td>
<td>6.80 ± 9.90</td>
<td></td>
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<tr>
<td>normal essential hypertension</td>
<td>1.35 ± 0.47</td>
<td>4.77 ± 6.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>2.32 ± 4.17</td>
<td>2.79 ± 3.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low renin</td>
<td>2.23 ± 1.91</td>
<td>9.47 ± 24.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>essential hypertension</td>
<td>4.79 ± 7.79</td>
<td>6.48 ± 6.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>1.69 ± 1.38</td>
<td>6.58 ± 5.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.37 ± 3.33</td>
<td>6.07 ± 5.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>0.98 ± 0.74</td>
<td>11.05 ± 10.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly higher than placebo pre-saralasin value (P < 0.05).
† Significantly lower than polythiazide pre-saralasin value (P < 0.05).
### Table 2: Subject Characteristics and Blood Pressures at Each Phase of Study

<table>
<thead>
<tr>
<th>Race</th>
<th>Sex</th>
<th>Placebo</th>
<th>Placebo and saralasin</th>
<th>Polythiazide</th>
<th>Polythiazide and saralasin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal renin</td>
<td>1</td>
<td>44</td>
<td>M</td>
<td>163</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45</td>
<td>M</td>
<td>163</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>42</td>
<td>B</td>
<td>146</td>
<td>110</td>
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<td></td>
<td>4</td>
<td>36</td>
<td>W</td>
<td>139</td>
<td>101</td>
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<td></td>
<td>5</td>
<td>43</td>
<td>B</td>
<td>152</td>
<td>113</td>
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<td>6</td>
<td>43</td>
<td>M</td>
<td>170</td>
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<td>9</td>
<td>36</td>
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<td>153</td>
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<td>Mean</td>
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<td></td>
<td>159</td>
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<td>160</td>
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<td>se</td>
<td></td>
<td></td>
<td>5.3</td>
<td>2.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Low renin</td>
<td>1</td>
<td>49</td>
<td>B</td>
<td>175</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>38</td>
<td>B</td>
<td>198</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>26</td>
<td>W</td>
<td>150</td>
<td>111</td>
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<td></td>
<td>4</td>
<td>36</td>
<td>W</td>
<td>186</td>
<td>112</td>
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<tr>
<td></td>
<td>5</td>
<td>38</td>
<td>B</td>
<td>176</td>
<td>105</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>177</td>
<td>118</td>
<td>177</td>
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<td>se</td>
<td></td>
<td></td>
<td>7.9</td>
<td>5.8</td>
<td>7.1</td>
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</tbody>
</table>

A diuretic-induced angiotensin-dependent component previously undemonstrable. However, in normal renin subjects, the decrease in blood pressure effect of polythiazide and saralasin was variable and did not normalize the mean pressure. A similar decrease did not occur in low renin subjects; in most, blood pressure rose persistently during both saralasin infusions.

**Relationship between Diuretic-Induced Change in Renin Activity and Change in Blood Pressure Response to Saralasin**

Changes in plasma renin activity in response to polythiazide and subsequent blood pressure responses to saralasin were variable. As shown in Figure 4, however, the mag-

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**Figure 2** The individual changes in diastolic blood pressure in response to saralasin in each renin group during placebo (open symbols) and diuretic therapy (filled symbols) are shown. The corresponding points in the upper and lower lines represent the same subject. Seven of nine normal renin subjects and only one low renin subject showed variable depressor responses to saralasin during chronic diuretic therapy.
RENIN RESPONSE LIMITING DIURETIC EFFICACY/Vaughan et al.

Discussion

Diuretic management usually is instituted in patients documented to have sustained hypertension; if the blood pressure does not normalize, sympatholytic agents usually are added. More recently, therapy based on measurements of peripheral plasma renin activity has been effective. 5, 3, 5, 11, 12

A more enlightened understanding of the physiological mechanism underlying a refractory response to diuretic treatment would allow a more rational choice of additional or alternative antihypertensive agents. Diuretic therapy enhances sodium and water loss; the ensuing volume contraction is sensed by the renal juxtaglomerular apparatus13 and renin is released, resulting in sustained elevations of plasma renin activity. 3 Such rises were observed in all of our subjects, but were less than those accompanying treatment with spironolactone or chlorthalidone. 2 Previously, the net cardiovascular effect of this rise has been difficult to determine because thiazide therapy1,1 and sodium depletion6 reduce vascular responsiveness to exogenous angiotensin II.

In sodium-deprived animals, studies with inhibitors of converting enzyme or angiotensin II receptor antagonists have shown that the reactive increase in renin secretion produces angiotensin dependence of blood pressure15 and renal function. 16 Moreover, rigid dietary sodium restriction enhanced by acute diuretic-induced natriuresis uniformly resulted in a saralasin depressor response in both low and normal renin hypertensives whose blood pressures were resistant to saralasin prior to sodium depletion. 8 These data suggest that the increased renin secretion induced by conventional chronic diuretic therapy alone might attenuate diuretic antihypertensive efficacy via angiotensin II-induced vasoconstriction. Indeed, blood pressures in seven of nine normal renin subjects fell with polythiazide, and diastolic pressures fell further (from 1 to 14 mm Hg) after the addition of saralasin. In four subjects, the two agents in combination reduced blood pressure to approximately 90 mm Hg. These individual responses and the correlation between the changes in plasma renin activity and in blood pressure responses to saralasin show that the compensatory rise in renin limits diuretic efficacy in some patients. Saralasin alone decreased diastolic pressure in only two of these subjects.

Further evidence for a physiological effect of circulating angiotensin II in our subjects was based on the angiotensin feedback suppression of renin release. 17 Saralasin-mediated inhibition of this feedback resulted in increased plasma renin activity in normal renin subjects only when the blood pressure fell in response to angiotensin blockade.

However, individual blood pressure responses to polythiazide were not accurate indicators of ensuing responses.
to saralasin. Inasmuch as decreased sodium induces a change in vascular responsiveness to angiotensin II and saralasin, it is not surprising that neither the blood pressure nor the absolute level of plasma renin activity correlated with the magnitude of the saralasin response during diuretic therapy. Individual blood pressure responses to these agents are more complex than responses to acute sodium depletion alone. Hence the magnitude of either the blood pressure response to diuretic alone or the diuretic-induced angiotensin II component was not constant. These individual differences are emphasized further by the failure of blood pressures in four normal renin subjects to normalize with simultaneous volume depletion and angiotensin blockade. Incomplete elimination of a volume component by chronic polythiazide therapy alone or the agonist activity of saralasin, which can compromise one's ability to quantitate an angiotensin II component of the blood pressure, could certainly explain the residual hypertension. Alternatively, one or more factors unrelated to volume excess or angiotensin II-induced vasoconstriction could contribute to the maintenance of blood pressure in some patients with normal renin essential hypertension.

Although the conditions of this and a previous study are different, the depressor effect of saralasin during diuretic administration in two normal renin essential hypertensives was the same as that previously used to identify "angiotensinogenic" hypertension (a diastolic fall > 7 mm Hg); in one of our patients, the effect was borderline (-14/-7). Hence, saralasin infusion in patients with essential hypertension on diuretic therapy can result in a "false positive" depressor response. However, the conclusion that patients should be tested by saralasin infusion only after discontinuation of diuretic therapy presents a potential problem. Some patients, usually those with low renin essential hypertension, display an alarmingly severe pressor response during saralasin infusion. Dietary or diuretic-induced sodium depletion protects the patient from this potentially dangerous response.

Thus, although chronic diuretic therapy limits the accuracy of a saralasin test to identify only patients with intrinsic, unstimulated, angiotensin-dependent blood pressure, acute furosemide pretreatment or an increasing rate of saralasin infusion may be necessary to avoid the hazard of further blood pressure elevation.

An alternative approach would be to administer saralasin selectively. Patients with fixed essential hypertension would be initially placed on diuretic therapy. Although diastolic blood pressures fell below 90 mm Hg in only two of our five low renin subjects, most investigators of such patients have found that 60-100% will normalize blood pressure in response to diuretics. In contrast, a pressor response to diuretics can occur in patients with renovascular hypertension. Hence, a clinical trial with diuretics would identify most low renin patients who do not require a saralasin challenge. The remaining patients, those refractory to diuretic therapy and also less likely to exhibit pressor responses to saralasin, could be evaluated subsequently for angiotensin dependency after cessation of diuretic therapy.

In contrast to the normal renin hypertensives, four of five low renin hypertensives exhibited pressor responses to saralasin after diuretic therapy. The reactive diuretic-induced rise in renin was small and probably did not contribute appreciably to blood pressure maintenance. This blunted renin response to the diuretic may be explained by the low basal renin level, less effective volume depletion, or decreased renin release in response to volume depletion. The fact that diastolic blood pressures normalized (<90 mm Hg) in two subjects on diuretic therapy who continued to exhibit pressor responses to saralasin would suggest that volume depletion was adequate without a renin response capable of limiting the therapeutic efficacy of polythiazide. The small diuretic-induced rise in renin, and possibly the low basal renin value, may represent decreased adrenergic stimulation of renin release, inasmuch as low dopamine-β-hydroxylase activity and plasma norepinephrine levels have been observed in low renin essential hypertensives. The one subject whose blood pressure decreased in response to saralasin demonstrates the heterogeneity of the low renin hypertensive population which was not distinguishable by the renin response to diuretic.

In summary, these studies indicate that the compensatory rise in plasma renin activity in response to diuretic-induced volume depletion limits therapeutic efficacy in some patients. In these patients, attenuation of the compensatory rise in plasma renin activity by a second antihypertensive agent, such as propranolol, should result in more effective blood pressure control. However, because of the complexity of the angiotensin II-sodium-receptor interaction in hypertensive patients, it remains difficult to predict factors responsible for blood pressure resistance during antihypertensive therapy in an individual patient.

Acknowledgments

We are indebted to Dr. Robert Kennan of Norwich Pharmaceuticals for supplying saralasin acetate.

References

ISOPROTERENOL-EVOKED RENIN RELEASE IN RATS/Sinaiko and Mirkin

Dose-Response Characteristics in Spontaneously Hypertensive and Normotensive Wistar Rats

ALAN R. SINAIKO AND BERNARD L. MIRKIN

SUMMARY We studied the dose-response relationship between renin secretion and isoproterenol administration in the in situ perfused kidney of spontaneously hypertensive (SHR) and normotensive Kyoto Wistar (WKY) rats obtained from two breeding facilities (BioLab and Taconic Farms). Following pulse injections of isoproterenol (doses ranging from 5 x 10^-11 to 5 x 10^-8 M), timed samples of perfusate were taken over a 30-minute period and perfusate renin activity (PeRA) in each sample was determined by radioimmunoassay. Analysis of regression lines constructed from data representing the renin secretory response (quantified by plotting PeRA of each sample against time of collection and measuring the area under the curve) to isoproterenol concentration demonstrated a positive dose-response relationship for WKY (y = 690.87 + 2864.06x, P < 0.05) and SHR (y = 206.56 + 1496.54x, P < 0.05) from BioLab and WKY (y = 138.35 + 74.39x, P < 0.05) from Taconic Farms. However, regression analysis of data obtained from studies in Taconic Farms SHR (y = 65.15 + 0.95x, P > 0.05) did not demonstrate a significant relationship between dose and response. Renin secretion was found to be significantly greater in both WKY strains when comparisons were made between the regression coefficients of WKY and SHR (y = 65.15 + 0.95x, P > 0.05). This investigation has established that the release of renin from the kidney elicited by isoproterenol follows a dose-response pattern similar to that observed with other adrenergically mediated responses. Furthermore, the response of the WKY to isoproterenol-evoked renin secretion was consistently greater than that observed in the SHR strains.

ACTIVATION of the renin-angiotensin system is regulated, in part, by β-adrenergic mechanisms. Intra-arterial administration of isoproterenol to the in situ perfused rat kidney,1,2 incubation of rat kidney slices in catecholamine-containing medium,3 and electrical stimulation of isolated canine renal nerves4 produce an increase in renin release that can be blocked by propranolol.

Traditionally, the reactivity of autonomic effector sys-
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