Angiotensin II, Plasma Renin and Sodium Depletion as Determinants of Blood Pressure Response to Saralasin in Essential Hypertension

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SUMMARY To evaluate the role of the renin-angiotensin system and sodium depletion in the hypotensive response to 1-sarcosine-8-alanine-angiotensin II (saralasin), 15 male patients with essential hypertension were studied on a diet containing 120 mEq of sodium and 100 mEq of potassium per day. After a 5-day control period, all subjects had a mild pressor response to the saralasin infusion (p < 0.01). After 5 days of the diuretic metolazone (5 mg/day), eight of the 15 patients had a vasodepressor response; these responders had a significantly greater increase in plasma renin activity and angiotensin II concentrations than did the non-responders. Sodium deficit differed markedly (p < 0.001) between the two groups (361 ± 121 mEq (SD) vs 52 ± 26 mEq sodium, respectively). The addition of spironolactone (400 mg/day) for 5 days resulted in saralasin responsiveness in all but two patients, both of whom had small sodium deficits. Thus, variability in the natriuretic response to diuretics may affect saralasin testing and limit its clinical utility.

THE DEVELOPMENT of competitive antagonists to angiotensin II (AII) has opened a productive avenue of research into the role of the renin-angiotensin system in normal physiology and blood pressure regulation. One of these compounds, 1-sarcosine-8-alanine-angiotensin II, or saralasin, has been extensively studied in humans and has been reported to be useful in identifying hypertensive patients in whom AII is the major cause of elevated blood pressure.1-5 However, saralasin has displayed evidence of agonistic as well as antagonistic activity. This agonist potential of the drug has prompted the claim that saralasin testing leads to an underestimation of the role of AII in most forms of hypertension6-7 and is believed to be the explanation for the hypertensive responses sometimes observed with its administration.8-10 Diuretic medication usually precedes saralasin infusion, as this procedure lessens the hypertensive reaction and enhances the hypotensive response.11 Although patients with low or normal plasma renin activity (PRA) generally do not display hypotensive responses to saralasin, prolonged or vigorous diuresis has been reported to result in positive tests.12,13 We studied the development of saralasin responsiveness during progressive sodium depletion with oral diuretics in patients with essential hypertension. The sodium deficit and changes in PRA and AII levels were found to be indicators of the development of the hypotensive response to saralasin.

Materials and Methods

Patients

Fifteen male patients ages 28-65 years with the diagnosis of essential hypertension were selected for the study from the outpatient hypertension clinic at the Sepulveda Veterans Administration Medical Center. Secondary causes of hypertension and the presence of significant target organ damage were excluded on the basis of a detailed clinical assessment, chest x-ray, ECG, serum creatinine and rapid-sequence, intravenous pyelography. Patients were taken off all medications for at least 4 weeks before the study.

Study Protocol

Patients were admitted to a clinical investigation ward and maintained on a constant daily diet containing 120 mEq of sodium and 100 mEq of potassium throughout the study. Urinary electrolytes in 24-hour urine collections were measured daily. Saralasin infusion was performed three times: after 5 days of the constant diet, after the addition of metolazone (5 mg daily) for 5 days, and after 5 days of the combined regimen of metolazone (5 mg/day) and spironolactone (100 mg four times a day).

Patients were in the supine position for 30 minutes before starting the infusion. Blood pressure was recorded at 2-minute intervals with an automatic ultrasonic device (Arteriosonde 1216, Roche Medical Electronics). Saralasin was infused in increasing doses of 0.1, 1.0, 4.4, 8.8 and 12 μg/kg/min at 12-minute intervals with an infusion pump (Harvard Apparatus, Inc.). The infusions were discontinued when diastolic pressure fell by 16 mm Hg or more. Blood was drawn for PRA, plasma renin substrate (PRS) and circulating AII determinations before and after the saralasin infusion.
Laboratory Measurements and Statistical Methods

Serum electrolytes and urinary creatinine, sodium and potassium were measured by automated techniques in the hospital clinical laboratory. For measurement of PRA, plasma was incubated for 60 minutes at 37°C at pH 7.4 in the presence of EDTA, DFP and 8-hydroxy-quinoline, as described by Barrett et al. The generated angiotensin I was measured by radioimmunoassay using a method previously reported. PRS concentration was determined by radioimmunoassay measurement of generated angiotensin I upon incubation with added excess homologous renin in the presence of angiotensinase inhibitors, according to the method of Eggena et al. Circulating AII was extracted from 20 ml of peripheral venous blood and measured by the method of Barrett et al. The cumulative sodium deficit achieved with diuretic administration was calculated by subtracting the total urinary sodium excreted during the control period from that of the experimental periods.

In view of the number of patients, the Wilcoxon nonparametric analysis was used for statistical evaluation. Results are expressed as mean ± sd. A probability of less than 0.05 was considered significant.

Results

Blood Pressure Response to Saralasin

A reduction of 8 mm Hg or more of mean supine blood pressure was defined as a positive response to the infusion of saralasin. During the control period, no vasodepressor responses were seen and, in fact, a slight pressor response was observed in all subjects, with an average increase of mean blood pressure of 4.0 ± 5.8 mm Hg (p < 0.01). With the one-diuretic regimen, eight of 15 patients displayed a vasodepressor response with a decrease in mean blood pressure of 15.0 ± 3.9 mm Hg. Patients who responded to saralasin did not differ from nonresponders with regard to age (51.2 ± 11.2 years vs 53.1 ± 10.4 years), weight (87.2 ± 16.1 kg vs 80.7 ± 11.8 kg) or creatinine clearance (81.1 ± 6.6 ml/min/1.73 m² vs 88.2 ± 7.6 ml/min/1.73 m²) (table 1). After two diuretics, 13 of 15 patients had a vasodepressor response with a fall of mean blood pressure of 18.0 ± 7.2 mm Hg.

During the saralasin infusions no significant changes in heart rate were seen in either nonresponders or responders. After two diuretics, one subject had a sudden, severe fall in mean blood pressure of 30 mm Hg while receiving saralasin at a dose of 8.8 ng/kg/min. When the infusion was stopped and intravenous saline administered, the blood pressure quickly returned to baseline.

PRA and Circulating AII

After metolazone, the eight responders increased their preinfusion PRA values from 5.9 ± 6.8 to 26.0 ± 13.3 ng/ml/hr (p < 0.05), while the nonresponders increased from 2.7 ± 3.2 to 6.8 ± 3.7 (p < 0.05). The increase over basal values of 20.1 ± 13.3 for the responders was significantly different from the increase of 4.1 ± 2.9 ng/ml/hr in the nonresponders (p < 0.05). Changes in circulating AII paralleled the changes in PRA. Among responders, circulating AII increased from 3.9 ± 3.9 to 30.0 ± 14.7 pg/ml after one diuretic (p < 0.05), and in the nonresponders circulating AII increased from 1.9 ± 2.6 to 8.2 ± 6.3 pg/ml (p < 0.01). The difference in the increase of circulating AII was significant (p < 0.05) (26.1 ± 17.5 pg/ml vs 6.3 ± 7.4 pg/ml).

PRA and AII levels obtained simultaneously before the saralasin infusions correlated strongly (r = 0.85; p < 0.001) (fig. 1). PRA values over 16 ng/ml/hr and AII levels greater than 20 pg/ml were generally associated with a vasodepressor response (fig. 1). There was no significant change in PRA after diuretic therapy in either responders or nonresponders (table 1).

The effect of saralasin infusions on PRA and AII levels was dependent upon the blood pressure response during the infusion. When there was no hypotensive

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Saralasin Responders and Nonresponders</th>
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<tr>
<td>Responders</td>
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<tr>
<td>One diuretic (n = 8)</td>
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<td>One diuretic (n = 7)</td>
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<tr>
<td>PRA (ng/ml/hr)</td>
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<td>AII (pg/ml)</td>
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<td>PRS (ng/ml)</td>
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<td>Creatinine clearance (ml/min/1.73 m²)</td>
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Values are mean ± sd.
Abbreviations: PRA = plasma renin activity; AII = angiotensin II; PRS = plasma renin substrate.
response, PRA values did not change significantly (from 5.6 ± 4.2 ng/ml/hr before the infusion to 6.2 ± 6.3 ng/ml/hr after the infusion). AII levels increased slightly, from 7.5 ± 8.7 to 14.3 ± 16.4 pg/ml (p < 0.01). In the positive responders, PRA increased from 32.0 ± 16.4 to 118.8 ± 96.3 ng/ml/hr (p < 0.001) and AII levels increased from 35.8 ± 16.2 to 93.6 ± 42.7 pg/ml (p < 0.001).

Sodium Deficit

After 5 days of metolazone, patients who responded to saralasin had a sodium deficit of 361.2 ± 121.3 mEq (4.4 ± 1.5 mEq/kg body weight). The nonresponders had a calculated sodium deficit of only 52.0 ± 26.2 mEq (0.6 ± 0.3 mEq/kg body weight) (table 1). This difference was statistically significant (p < 0.001). After the addition of spironolactone, the cumulative sodium deficit was 546.7 ± 164.4 mEq in the 13 responders. The two nonresponders had sodium deficits of 60.0 mEq and 186.5 mEq. A sodium deficit of greater than 225 mEq was always associated with a saralasin vaso depressor response. Sodium deficit was linearly related to changes in PRA (r = 0.53; p < 0.001) (fig. 2). This relationship also held when the sodium deficit was corrected for body weight (r = 0.48), body surface area (r = 0.48) or body mass (weight in kg/[height in cm]^3) (r = 0.47) (all p < 0.001).

Discussion

The angiotensin II antagonist saralasin has been administered by continuous infusion and bolus injection to normal subjects and patients with hypertension. Without prior diuretic administration, saralasin does not give a hypotensive response in a significant fraction of patients with renovascular hypertension. Marked hypertensive responses to saralasin have been noted in subjects with "low-renin" hypertension who have not been sodium-depleted. For these reasons it has appeared advantageous to treat patients with diuretics before saralasin administration, although the optimum protocol for diuretic usage is not clear. Anderson and co-workers placed their hypertensive subjects on 4–5 weeks of oral diuretics and found that even some of these "low-renin" patients had a hypotensive response to saralasin. Similarly, Gavras et al. found that vigorous diuretic therapy could produce a hypotensive saralasin response in most essential hypertensives.

The findings in this study are in agreement with the concept that the degree of sodium depletion is critical in determining the response to saralasin. After the addition of spironolactone to the metolazone induced diuresis, only two of 15 subjects failed to have a fall in pressure during the saralasin infusions. These two patients both had cumulative sodium deficits of less than 200 mEq, suggesting a suboptimal natriuresis. This relationship was even more striking after the single-diuretic period. The eight responders to saralasin after one diuretic displayed a sodium deficit that was more than seven times that of the seven nonresponders.

Gavras et al. used a low-sodium diet and hydrochlorothiazide to achieve sodium depletion and did not observe significant differences in sodium balance in their groups, though the responders averaged a greater sodium deficit than the nonresponders. Marks et al. also measured sodium excretion after furosemide (1 day) and found a slight but significant difference, with their responders excreting an average of 170 mEq of sodium compared with an average of 129 mEq of sodium in the nonresponders. These values overlapped considerably, and there was a poor relationship between sodium deficit and changes in PRA. This latter finding may be partially explained by the ability of furosemide to stimulate renin release through mechanisms besides its natriuretic effect. In the present study, however, the differences in sodium deficit between responders and nonresponders were marked, and the relationship between sodium deficit and changes in renin activity was highly significant (fig. 2). This variability in sodium excretion was not attributable to any differences in age, weight or glomerular filtration rate (table 1).
One patient had a marked hypotensive response to saralasin after sodium depletion with two diuretics. This patient had a severe deficit of 988 mEq of sodium. Blood pressure was quickly restored when the infusion was discontinued and saline administered. Thus, caution is required in the use of saralasin after diuresis. Hypotension with saralasin has also been reported in patients taking vasodilator drugs\(^2^9\) and in patients with cirrhosis.\(^2^0\) The hypertensive response in this patient and the other responders was unaccompanied by a change in the pulse rate. The hypertensive response to saralasin in man has been attributed to changes in cardiac output as well as peripheral resistance.\(^2^1\)

The responses of the renin-angiotensin system in this study are in accord with previous investigations that have correlated the hypotensive reaction to saralasin with changes in PRA.\(^2^2\),\(^2^3\) PRS did not change significantly during the study.

Because saralasin is felt to act through its competition with AII at vascular receptors,\(^2^4\) the response to this drug will be determined, in large part, by the level of circulating AII, as observed by Brown et al.\(^2^5\) in a series of normal subjects and patients with hypertension of various etiologies. Our results support the validity of the measurement of circulating AII in predicting a hypotensive saralasin response. However, the linear relationship between PRA and circulating AII was very strong, and the technically easier PRA assay was equally predictive under the conditions of this study. While this result was anticipated, it is nonetheless important, because the PRA-AII relationship does not always hold true.\(^2^6\)

The saralasin responders showed a marked increase in both PRA and AII during the infusions. This increase could represent either stimulation mediated by baroreceptor reflexes or blockade of renin-suppressive AII intrarenal receptors, or both.\(^1^9\) The significant rise in AII levels accompanying unchanged PRA values in nonresponders may be explained by very slight cross-reactivity of saralasin in the AII assay, although this was not detected in vitro.

Because the changes in plasma renin were related to the sodium deficit (fig. 2), it is not possible to ascribe, with certainty, an independent role for sodium depletion in the saralasin response. However, a saralasin hypotensive response could be predicted better from the sodium deficit than from the preinfusion levels of either PRA or AII. One of the two subjects who did not respond to saralasin after two diuretics had elevated PRA and AII levels, but a small sodium deficit. Lack of a saralasin response has been noted in volume expanded diastolic patients with high PRA.\(^2^7\) These observations suggest that sodium and volume depletion affect the saralasin response through mechanisms additional to the circulating AII level.

The 15 patients studied in this report responded similarly to saralasin during the control period. All the patients had a discernible rise in mean blood pressure that did not exceed 10 mm Hg. Gavras et al.\(^1^2\) reported that four of their 15 patients had significant pressor responses averaging 24 mm Hg, even after volume depletion. Vaughan et al.\(^2^8\) also reported that a pressor response after diuretic administration was seen in four of five patients classified as “low renin.” The pressor response in these patients averaged only 5 mm Hg, however. On the other hand, Marks et al.\(^1^9\) reported that three of their 15 patients with hypertension had a vasodepressor response before furosemide. These three were among the four patients who had been classified as having “high-renin” essential hypertension. None of the 15 subjects in the present study showed a vasodepressor response before diuretic administration. The hope that saralasin can be helpful in categorizing hypertensive patients into low-, normal- and high-renin groups requires further clarification.

The usefulness of saralasin as a tool in the diagnosis of renin-dependent hypertension will rest on the percentage of false-negative and false-positive results in clinical testing. A recent survey found this procedure to be an inadequate screening test for renovascular hypertension.\(^2^9\) While it is clear that prior diuresis helps to eliminate false-negative responses, many essential hypertensives will respond if they lose enough sodium. In the present study in male patients with essential hypertension, a sodium deficit greater than 225 mEq was always associated with a hypotensive response. However, there was great variability in the natriuretic response to the same dosage of diuretic. This difference in patient response could be an important variable in saralasin testing.

Acknowledgment

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