The Immediate Pressor Response to Saralasin in Man
A Test of Angiotensin II Receptor Vacancy

JOHN M. WALLACE, DAVID B. CASE, JOHN H. LARAGH, HANS J. KEIM, J. I. M. DRAYER, AND JEAN E. SEALEY

SUMMARY Saralasin, 10 μg/kg per min, caused an immediate rise in blood pressure in 52 of 57 (91.2%) hypertensive patients. The increase in diastolic pressure averaged 18.8 ± 1.83 mm Hg (mean ± SE) in normal renin patients on a normal salt intake. This immediate pressor response was absent in only five high renin patients and, conversely, was very large in three low renin patients. Direct arterial recordings are necessary to define the response accurately; it begins in 60-90 seconds, peaks in amplitude at 2.05 ± 0.38 minutes, and subsides over the next 5 minutes in normal renin and high renin patients. The blood pressure elevation is inversely related to background plasma renin activity (r = −0.66, P < 0.001), and also is directly, but weakly, related to 24-hour urinary sodium excretion (r = +0.29). Therefore, the amplitude of the elevation is predictably diminished by the rise in plasma renin consequent to prior sodium restriction, and also by preliminary receptor exposure to low dose nonpressor infusions of saralasin itself (0.01-0.1 μg/kg per min). Phentolamine had no effect on the response in two patients. We propose that the immediate pressor response to saralasin is related directly to the preexisting degree of vacancy of angiotensin II vascular receptors and that the initial agonistic action of the drug may prove useful in defining the angiotensin II receptor status in hypertensive diseases.

Circ Res 44: 38-44, 1979

SARALASIN (Sar¹-Ala²-angiotensin II) is a partial agonist with significant pressor activity in animals (Pals et al., 1971; Johnson and Davis, 1973) and man (Streeten et al., 1975; Case et al., 1976). However, its antagonism of angiotensin II (A II) rather than its intrinsic agonism has attracted more clinical attention because the former property enables saralasin to lower blood pressure in high renin hypertension (Streeten et al., 1975; Case et al., 1976; Brunner et al., 1973). Hollenberg et al. (1976), our group (Case et al., 1976), and Anderson et al. (1977) have shown that the intrinsic agonistic activity also has clinical relevance. Our data suggest that this activity not only accounts for the sustained pressor effect of saralasin in low renin patients, but also leads to an underestimation of the magnitude of the renin factor in high renin hypertension (Case et al., 1976). These characteristics, which are apparent during the sustained response to saralasin between the 10th and 30th minutes of a continuous infusion, reveal the operation of an agonistic action at a time when, were the drug only an antagonist, it would either lower the blood pressure even more profoundly in high renin patients, or would have no intrinsic pressor effect in the absence of any endogenous renin participation.

The present studies focus on a new agonistic action of saralasin, namely, an immediate and usually transient pressor effect which appears within 60-90 seconds of the start of infusion and is best defined using continuous intra-arterial recordings. This phenomenon occurred in 91% of our hypertensive patients and was quite marked in some patients with low renin hypertension who would be expected to have the highest degree of A II vascular receptor vacancy. The data suggest that the amplitude of the immediate pressor response is a measure of the initial degree of vacancy of these vascular receptors. This interpretation further suggests that the immediate agonistic action of saralasin might be used to determine the A II receptor vacancy-occupancy status in diverse hypertensive patients. Thus, we have found it useful in predicting the renin subtype of such patients.

Methods

Sixty-seven studies were performed in 57 hypertensive patients previously determined to have high renin, normal renin, or low renin-sodium profiles (Laragh et al., 1972). They had not received any antihypertensive medications for at least 21 days. The etiology of the hypertension was mixed and included 32 patients with normal renin essential
hypertension, 16 patients with high renin hypertension of varying etiology, and nine patients with low renin essential hypertension.

A 24-hour urine collection was completed on the morning of study and a corresponding plasma renin sample was drawn just prior to saralasin infusion. Plasma renin activity (PRA) was measured by the method of Sealey and Laragh (1975). All subjects were tested in the seated position except for 11 high renin and two low renin patients who were recumbent. In most subjects, saralasin was infused into a hand vein at a rate of 10 μg/kg per min for 30 minutes, exceptions being noted in the Results. Blood pressure was recorded at 2-minute intervals by Arteriosonde in 30 studies and by continuous intra-arterial measurement in 37 others. In studies with the Arteriosonde, neither the onset of the immediate pressor response nor the time and height of its peak could be determined precisely. Nevertheless, of the two or three pressures obtained by this method within the first 5 minutes of infusion, the highest one was selected and combined with data from the intra-arterial studies for most analyses. Intra-arterial data alone were used for defining the time course of the response. Diastolic blood pressures only are analyzed in this paper.

Informed written consent was given by each subject, and the study protocol was approved by the institutional committees. Results are expressed as mean ± SEM. As is appropriate in small samples, often without a normal distribution, nonparametric methods were used to assess differences between two groups (Wilcoxon’s two-sample signed rank test) and to calculate correlation coefficients between variables (Spearman’s correlation coefficient).

Results

Characteristics of the Immediate Pressor Response

In 52 of 57 studies in which only the standard infusion of saralasin of 10 μg/kg per min was employed, an immediate pressor response occurred. It was absent only in five subjects with high renin hypertension. In 19 studies with continuous intra-arterial recordings in normal renin subjects, the blood pressure rose within 60–90 seconds of the start of infusion, reached its peak at 2.05 ± 0.38 minutes, and returned to the baseline or remained slightly above it by the 6th minute (Fig. 1), a termination point that was characteristic of normal renin patients on a normal salt diet.

The course was different in low renin hypertension. In two subjects, diastolic blood pressure rose to 21% and 33% above control and the infusion was stopped at 3 and 5 minutes, respectively. The first subject, who received saralasin, 20 μg/kg per min, developed premature ventricular contractions; the second patient experienced headache. Symptoms in both ceased as the blood pressure declined. A third subject was given a dose of saralasin of only 1 μg/kg per min, but the diastolic pressure increased from 123 to 153 mm Hg by the 3rd minute and the infusion was stopped. In another low renin subject given 1.0 μg/kg per min, diastolic pressure immediately increased 24.2% but then slowly declined to a lower level, still above the baseline, without causing symptoms or other adverse effects. After these experiences, low renin subjects were tested first with infusion rates of 0.01–1.0 μg/kg per min which minimized the initial pressor effect.

Cardiac slowing accompanied the pressor response. In 25 studies with intra-arterial tracings, diastolic blood pressure rose from 87.0 ± 3.5 to a peak of 104.3 ± 4.0 mm Hg (P < 0.0005) and the heart rate fell from 82.7 ± 2.6 to 77.2 ± 2.1 beats/min (P < 0.005). Individual changes in blood pressure and heart rate were not correlated.

Effect of Baseline Plasma Renin Activity on the Immediate Pressor Response

In 52 studies the peak immediate pressor response to an infusion of saralasin of 10 μg/kg per min was inversely correlated with the initial level of PRA, r = −0.66 (Fig. 2). The regression line of this analysis intercepts the y axis (zero blood pressure increase) at a PRA of 16.2 ng/ml per hr, the theoretical background PRA above which no immediate pressor response should occur with the saralasin infusions used here.

Analysis by Renin Profiling

Because there is wide scatter about the regression line (Fig. 2), the pressor responses also were analyzed according to renin-sodium profiling (Fig. 3), differences between normal and low salt patients being indicated by (+). The responses were greater in normal renin hypertension than in high renin hypertension (P < 0.001), and greater in low renin hypertension than in normal renin hypertension (P < 0.001), as well as the peak response (P < 0.05), was reduced by salt restriction.

**Figure 1** The course of the pressor response during the first 5 minutes of saralasin infusion in normal renin hypertensive subjects. The overall response (all points analyzed, P < 0.001), as well as the peak response (P < 0.05), was reduced by salt restriction.
Immediate increase (%) in diastolic blood pressure

FIGURE 2. The relationship between the peak immediate pressor response (abscissa) and the baseline plasma renin activity (ordinate log) in all subjects. The log formula for the regression line is given here and in Figure 5.

than in normal renin hypertension, although the latter difference was not statistically significant, probably because some low renin subjects received small or abbreviated infusions to avoid excessive pressor effects.

Effect of Preliminary Low Dose Nonpressor Infusions of Saralasin

Nine normal renin subjects on a normal salt intake were given small infusions of saralasin (0.01–0.1 µg/kg per min) for 30 minutes. These infusions did not elevate blood pressure, but the standard infusion then caused a pressor response of only 7.3 ± 2.5% compared with 20.9 ± 2.0% (P < 0.01) in 18 other normal renin subjects not first given the smaller infusions. The standard infusion had no pressor effect at all in five other normal renin subjects who were both salt depleted and preinfused with nonpressor amounts of saralasin (Fig. 4).

Effect of Sodium Balance Possibly Independent of Renin

Forty-three studies were performed in subjects with normal salt intake who had a 24-hour urinary sodium excretion (UNaV) of 110.1 ± 15.4 mEq. Twenty-four studies were done in subjects receiving a low salt intake who had a UNaV of 16.6 ± 7.4 mEq. In all of these studies analyzed together, there

was a weak positive correlation between the salt intake as reflected by the UNaV and the immediate pressor response, $r = +0.29$. Six subjects were studied during both normal and low salt intake and all

FIGURE 3. The effect of dietary sodium depletion on the baseline plasma renin activity, upper panel, and the immediate pressor response, lower panel, in the three separate renin groups. Among these groups as a whole, the pressor responses were greater in normal renin than in high renin subjects (P < 0.001). Most low renin subjects received smaller saralasin infusions (0.1–1.0 µg/kg per min) or the infusions were stopped in less than 5 minutes, so a valid statistical comparison of this group with the others cannot be made.

FIGURE 4. Reduction of the immediate pressor response to saralasin, 10 µg/kg per min, by smaller prior infusions of saralasin.
had intra-arterial blood pressure recordings (Table 1). In these six paired studies, the pressor responses correlated equally with the control PRA, $r = -0.67$, and the UNaV, $r = +0.63$, which is to be expected because of the high correlation between log UNaV and log PRA, $r = -0.93$.

**Effect of Pretreatment with Phentolamine**

Pressor responses to angiotensin analogues in rats have been ascribed to release of adrenal catecholamines (Sen et al., 1974; Múnoz-Ramírez et al., 1975). Accordingly, two subjects with diastolic pressure responses to saralasin of +17% and +23% were given saralasin again 90 seconds after phentolamine, 5 mg, iv. The repeat pressure increments were +32% and +20%, respectively, having been undiminished by phentolamine. In addition, if adrenal epinephrine were released, one might expect the heart rate to increase as the pressure rose during the first 2 minutes of infusion. However, as noted above, heart rate fell during this period.

**Discussion**

The present study reports and characterizes in detail an immediate and usually transient pressor action of saralasin in man. Its rapid onset indicates that vasoconstriction begins with the first arterial passage of saralasin, 60-90 seconds after starting the infusion in a hand vein.

**Influence of Background Plasma Renin Activity**

Our data show that the initial agonistic action of saralasin depends substantially on the background PRA. When PRA was very high, absence of the effect was the rule, and such a result may even be expected when the PRA exceeds approximately 16 ng/ml per hr (Fig. 2). Conversely, in low renin hypertensive subjects, large immediate pressure elevations still occurred with a 10-fold reduction in dosage. The only subjects who displayed no immediate pressor responses were five high renin subjects, whereas only low renin subjects gave large responses. The response was diminished in all subjects when PRA was elevated by sodium restriction. These effects of saralasin are reminiscent of those of infused A II, the pressor response being inversely related to the PRA (Silah et al., 1967).

The initial pressor response and the sustained responses previously reported (Case et al., 1976) are both related to the PRA and are, therefore, related to each other. Large initial pressor responses predict small or absent subsequent depressor responses and vice versa, $r = +0.61$ (Fig. 5).

**Influence of Sodium and Catecholamines**

The wide scatter about the regression line relating immediate pressor responses to the control PRA suggests that other factors also are important. Two possibilities are considered here, an underlying sodium effect and the release of catecholamines by saralasin. For all subjects, a weak positive relation-

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**Table 1**

**Effect of Dietary Sodium Depletion** on the Immediate Pressor Response to Saralasin in Six Subjects Tested on Both Normal and Low Salt Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>DBP (mm Hg)</th>
<th>% increase in DBP with saralasin</th>
<th>UNaV (mEq/24 hr)</th>
<th>PRA (ng/ml per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt</td>
<td>92.7 ± 7.5</td>
<td>25.5 ± 3.4</td>
<td>135.2 ± 33.1</td>
<td>1.65 ± 0.2</td>
</tr>
<tr>
<td>Low salt</td>
<td>83.2 ± 5.4</td>
<td>15.4 ± 2.2</td>
<td>15.8 ± 6.8</td>
<td>6.78 ± 1.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

* One subject also received diuretics. DBP = diastolic blood pressure; UNaV = urinary sodium excretion per 24 hours. PRA = plasma renin activity.
ship between the pressor effect and the UNaV is demonstrable. However, the reciprocal relationship between UNaV and PRA, as shown in the six subjects with paired studies (Table 1), makes assessment of sodium balance as an independent variable impossible from the present data. It should be mentioned that our subjects were not salt loaded, the salt replete observations actually being made at a somewhat low normal UNaV of 110.1 ± 15.4 mEq. Sodium or volume as independent variables might be more readily judged if observations on higher salt intakes were available.

Catecholamine release could contribute to the immediate pressor response. In the studies of Múnoz-Ramírez and co-workers (1975) in rats, pretreatment with phenoxybenzamine and adrenalectomy both reduced the pressor response to saralasin at 2 and 10 minutes of infusion and blocked it at 30 minutes, a result attributed to catecholamine blockade or absence of catecholamine release. We suggest that a higher background level of PRA (not measured) in the adrenalectomized and phenoxybenzamine-treated animals may be an alternative explanation. Our results with phentolamine do not favor norepinephrine participation in the immediate pressor response in the two subjects tested.

Pressor Effect, Renin, and Receptor Availability

In the doses we used, saralasin behaved immediately like an agonist, most clearly when PRA was low and UNaV was normal or high. Pressor sensitivity to A II itself is also increased in low PRA-high sodium states, and several possible explanations have been reviewed (Thurston, 1976; Devynk and Meyer, 1976). (1) The affinity of the agonist for A II vascular receptors may be increased by sodium (Williams et al., 1976), and A II may be inactivated at a slower rate in animals on a high salt intake (Slack and Ledingham, 1976). (2) The number of A II vascular receptors may be increased in response to the low PRA accompanying high sodium intake (Devynk and Meyer, 1976). In what may be a related observation, Slack and Ledingham (1976) found that a period of 7 days was required for a large change in dietary sodium to produce a change in sensitivity to injected A II in the rat. This might be interpreted as a measure of the time required for synthesis or atrophy of vascular receptors. (3) Finally, the degree of vacancy of A II receptors may be increased when PRA is low.

We believe our data best support the receptor vacancy interpretation, although concomitant changes in receptor number or affinity for saralasin cannot be ruled out. First, the immediate pressor response is better related to background PRA than to UNaV in our subjects as a whole. Second, the potentially antagonistic phase of the action of saralasin is fundamentally related to the level of PRA and to the apparent degree of occupancy of the vascular receptors (Case et al., 1975). Therefore, it is reasonable to postulate that the preceding agonistic action of saralasin is related to the degree of underlying receptor vacancy. This interpretation is further supported by the finding that preliminary low dose nonpressor infusions of saralasin, which we presume undetectably occupy the vacant receptors in advance, reduce (salt replete) or abolish (salt deplete) the immediate pressor response to a large infusion (Fig. 4). Third, Thurston (1976) has shown that injection of the A I-converting enzyme inhibitor, SQ20881, into salt-depleted and adrenalectomized rats (high renin) leads to an immediate increase in the pressor response to A II. The immediacy of this effect favors increased vascular receptor vacancy after converting enzyme inhibition as the mechanism of augmented A II responsiveness. We presume the same result would obtain with the immediate pressor response to saralasin. A II generation within the arterial wall might also participate in a possible vacancy-occupancy mechanism as proposed by Swales et al. (1975).

The increased pressor sensitivity to A II or to the agonistic action of saralasin displayed by low renin or salt replete patients might have been interpreted earlier as due to volume expansion per se (Ames et al., 1965; Ishii et al., 1974). More recently, however, the effect of sodium administration on A II sensitivity has been viewed as working via an effect on endogenous renin or A II levels (Thurston, 1976; Ishii et al., 1974; Thurston and Laragh, 1975; Dehenneffe et al., 1976; Cowley and Lohmeier, 1978), on A II vascular receptor affinity (Williams et al., 1978; Slack and Ledingham, 1976; Kisch et al., 1976), or on receptor number (Devynk and Meyer, 1976), rather than through a nonspecific volume-dependent effect.

The receptor vacancy-occupancy hypothesis of the blood pressure effects of administered A II has gained recent support. It has been reviewed by Davis (1975) and Devynk and Meyer (1976), and advanced in particular by Thurston (1976; Thurston and Laragh, 1975). It has been employed by Dehenneffe and co-workers (1976) to account for differences between the A II-pressor dose response curves of normal and anephric subjects, and by Cowley and Lohmeier (1978) to explain volume-related changes in A II sensitivity in dogs. Investigations of these receptor mechanisms are complicated by the possible ability of arterial walls to synthesize A II (Thurston, 1976, Swales et al., 1975), by the slow dissociation of A II from vascular receptors in vitro (Rioux et al., 1976), and by the persistence of renin activity in vascular tissue for as long as 6 hours after nephrectomy (Rosenthal et al., 1969).

Saralasin and Receptor Theory

We have analyzed our interpretation of the behavior of saralasin as a partial agonist in terms of receptor theory. In low renin patients, saralasin appears to be an agonist whose intrinsic activity is less than maximal (less than A II) but not zero...
The reduced intrinsic activity of saralasin is probably due to the substitution of alanine in position 8 (Regoli et al., 1974). Because a partial agonist requires a high degree of receptor availability to exert a potent agonistic response (Rang, 1986), it seems likely that receptor availability or vacancy is great when saralasin is highly pressor. Furthermore, to occupy a large fraction of the receptor pool, partial agonists must be given in high concentration and, therefore, they act rapidly (Waud, 1968). The actions of saralasin we have observed are in keeping with these expectations.

The kinetic model of drug-receptor interactions suggests that the ultimate potency of a competitive antagonist will be greater when the agent acts slowly (Waud, 1968; Paton, 1961). In the case of the partial agonist, saralasin, the agonistic property may be expressed first, given enough vacant receptors and excess drug, because the rapid rate of association (k1) is large of drug with available receptors is stimulatory (Paton, 1961; Goldstein et al., 1974). However, the rate of dissociation from the receptors is presumably slower for saralasin than for A II (k2 for saralasin presumably smaller than for A II), probably because of replacement of Asp by Sar in position 1 (Regoli et al., 1974; Goodfriend and Peach, 1975). This substitution results in high affinity of the peptide for receptors and in marked tachyphylaxis, both of which may be increased by resistance to the activity of angiotensinases (Hall et al., 1974; Moore and Khairallah, 1976) and by tight binding with reduced displacement of peptide from superficial calcium-binding sites on the smooth muscle cells (Paiva et al., 1977). If such factors do, in fact, give saralasin a smaller k2 than A II, then the slower overall rate of drug-receptor interactions of saralasin would be expected to antagonize A II. Therefore, when the background renin level is within the range which allows for the expression of both agonism and antagonism, the agonistic expression is immediate (maximal in 2 minutes), whereas the antagonistic expression is delayed, although sustained, requiring 10-20 minutes to equilibrate (Case et al., 1976).

**Saralasin Testing**

The present study expands the potential usefulness of saralasin testing in hypertension. A large pressor response in 2 minutes is positive for patients with low plasma renin and many vacant receptors, whereas a large depressor response at 10 minutes and thereafter is positive for patients with high plasma renin and many occupied receptors. Between these extremes, when the ratio of vacant-to-occupied receptors is more balanced, one should expect saralasin responses to be difficult to employ clinically. In patients with unknown renin status, small infusions of saralasin of 1.0 μg/kg per min or less should be used for initial testing to avoid possible large immediate pressor responses. Finally, our data were obtained in untreated hypertensive subjects. Whether saralasin can be applied to testing for A II receptor vacancy in treated subjects has not been determined.

**References**


Pals DT, Masucci FD, Denning GS Jr, Sipos F, Fessler DC: Role of the pressor action of angiotensin II in experimental hyper-

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Carotid Sinus Reflex Coronary Vasoconstriction during Controlled Myocardial Oxygen Metabolism in the Dog

JAMES R. POWELL AND ERIC O. FEIGL

SUMMARY We studied carotid baroreceptor reflex coronary vasoconstriction in closed-chest dogs with controlled aortic blood pressure and myocardial oxygen metabolism. The dogs were anesthetized with morphine and chloralose. Left coronary blood flow was measured by a cannula-tip flowmeter, and myocardial oxygen metabolism was calculated by measuring the arterial-coronary sinus oxygen difference. A bilateral vagotomy was performed in all animals and they were treated with propranolol. Reduction of carotid sinus pressure to 40 mm Hg caused an increase in aortic pressure that was limited to 15 mm Hg by means of a pressure-control reservoir. During carotid hypotension, heart rate and myocardial oxygen metabolism were unchanged but diastolic coronary vascular resistance increased by 21%. Intracorony artery infusion of norepinephrine had similar effects. After interruption of the reflex arc with the a-adrenergic receptor antagonist, dibozane, carotid sinus hypotension and the same increase in aortic pressure (15 mm Hg) resulted in only a 5% increase in diastolic coronary resistance. Dibozane also reduced the coronary responses to norepinephrine. It is concluded that carotid sinus hypotension results in reflex sympathetic a-receptor coronary vasoconstriction and that this reflex vasoconstriction is independent of changes in myocardial oxygen metabolism or changes in aortic pressure. Circ Res 44: 44-51, 1979

A PREVIOUS study from this laboratory (Feigl, 1968) and investigations by Disalvo et al. (1971) and Limet et al. (1975) demonstrated a reflex coronary vasoconstriction in response to carotid sinus hypotension. Kirchheim (1976) has questioned the interpretation of the results obtained in the previous study by Feigl (1968), pointing out that they might be explained by coronary autoregulation (relatively constant flow despite changing perfusion pressure). In the previous study, both calculated diastolic coronary vascular resistance and aortic pressure increased during the carotid sinus reflex in animals treated with propranolol. The increased coronary resistance was abolished by cardiac sympathectomy, but the reflex rise in aortic pressure was reduced as well. Thus, the difference in the
The immediate pressor response to saralasin in man: a test of angiotensin II receptor vacancy.

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Circ Res. 1979;44:38-44
doi: 10.1161/01.RES.44.1.38

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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