The immediate pressor response to saralasin in man: evidence against sympathetic activation and for intrinsic angiotensin II-like myotropism

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Summary

1. The cardiovascular and hormonal effects of intravenous saralasin (0.5, 1 and 5 μg min⁻¹ kg⁻¹) were assessed in nine tetraplegic patients (with complete cervical spinal cord transaction above the sympathetic outflow) and in six normal subjects.

2. In the tetraplegic patients, saralasin caused an immediate transient pressor response which was not dose-dependent and substantially greater than the pressor response in normal subjects. The pressor response in the tetraplegic patients was not accompanied by a rise in levels of plasma noradrenaline.

3. In the tetraplegic patients, after α-adrenoceptor blockade with thymoxamine (1 mg kg⁻¹ h⁻¹), twice the dose of intravenous noradrenaline was needed to induce the same pressor response. The pressor response to saralasin (5 μg kg⁻¹ min⁻¹), however, was unaffected by thymoxamine.

4. Saralasin caused minimal changes in levels of plasma renin activity and plasma aldosterone in both groups. There was no relationship between basal plasma renin activity and the pressor response in either group.

5. We therefore conclude that the immediate transient pressor response to saralasin in man is not due to central sympathetic stimulation, is unlikely to be due to peripheral sympathetic activation and is probably the result of intrinsic angiotensin II-like myotropism.

Key words: angiotensin II, catecholamines, renin, saralasin, sympathetic nervous system, tetraplegia.

Introduction

There is controversy in man about the mechanisms of the immediate pressor response to intravenous saralasin. Some groups favour partial agonism simulating angiotensin II, largely on the basis of the inverse relationship between basal plasma renin activity and the pressor response to saralasin [1, 2]. Other workers, however, have argued for an interaction with the sympathetic nervous system, because of the close relationship between changes in plasma noradrenaline levels and blood pressure [3]. To investigate further the mechanism of this response, which has both clinical and physiological implications, we infused saralasin into tetraplegic patients with complete cervical spinal cord transections, who do not appear to have cerebral control over the peripheral sympathetic nervous system. To further exclude the participation of sympathetic activation, the infusions were repeated after the α-adrenergic blocker thymoxamine. Comparisons were made with a group of normal subjects.

Subjects and methods

Nine chronically injured tetraplegic patients (seven males and two females) aged between 21 and 43 years with physiologically complete, stable, cervical spinal cord transection between C4 and C8.
were studied. None of the patients had been on drugs for at least 4 days before the investigations, and all were on unrestricted diets. Six patients had urinary catheters which facilitated emptying of the bladder. Biochemical indices of renal function were normal in all patients and there was no evidence of systemic disease. Limited studies were also performed on a recently injured tetraplegic patient in 'spinal shock'. He had a complete C4/C5 lesion of 4 weeks' duration with diaphragmatic paralysis and was on a respirator. Physiological testing using previously described techniques [4] indicated absence of even isolated spinal cord sympathetic reflex activity.

Six healthy normal subjects (four males and two females) aged between 21 and 30 years, who were familiar with clinical investigative procedures, were studied as controls. None was on drug therapy or dietary restriction. Ethical permission for the studies was obtained from both hospitals and each subject gave informed consent.

In the tetraplegic patients, blood pressure was measured by using an intra-arterial catheter (Abbocath 16G) introduced percutaneously. This was connected to a pressure transducer maintained at the level of the right atrium and the signal was fed into a four-channel pen recorder (Devices M4). Heart rate was derived from the electrocardiograph signal via surface electrodes and was displayed on the multi-channel recorder. A three-way connector attached to the arterial cannula facilitated withdrawal of blood for the measurement of plasma noradrenaline (radioenzymatic assay [5]), plasma renin activity (radioimmunoassay [6]) and plasma aldosterone (radioimmunoassay [7]). A cannula was also inserted into a peripheral vein for drug infusion. Saralasin (Eaton-Norwich Laboratories) was diluted in 5% glucose solution and infused in three doses of 0.5, 1 and 5 μg min⁻¹ kg⁻¹ with a calibrated Harvard infusion pump. Noradrenaline (Levophed, Winthrop) and thymoxamine (Opilon, Warner-Lambert) were freshly diluted in sodium chloride solution (0.5 mol/l) and administered with a Meltec infusion pump. In the normal subjects, blood pressure was measured by using an automated sphygmomanometer (Dinamap 845) at 5 min intervals, except during the first 10 min of infusion when it was measured after 3, 5, 8 and 10 min. Blood from a cannula in a forearm vein was collected for measurement of plasma renin activity and plasma aldosterone.

The protocol consisted of insertion of the cannula, after which the subject rested for at least 1 h before the base-line measurements. Care was taken to ensure that the bladder was empty and that continuous drainage occurred in patients with urinary catheters, so as to prevent spinal sympathetic reflex activity [8]. Two blood samples, 5 min apart, were collected for basal measurement of plasma renin activity and plasma aldosterone. Each dose of saralasin was infused for 20 min. Plasma noradrenaline was measured before and 3 and 5 min after the start of infusion while plasma renin activity and aldosterone were measured at the end of infusion and 5 min later. There was an interval of approximately 80 min between infusions.

After the three saralasin infusions, the α-adrenoceptor blocker thymoxamine (1 mg h⁻¹ kg⁻¹) was intravenously infused for 30 min, after which saralasin (5 μg min⁻¹ kg⁻¹) was then re-infused for a period of 10 min. This dose of thymoxamine had been previously used safely in tetraplegic patients [9] and, as will be described, was effective in antagonizing the pressor response to infused noradrenaline. In six patients the pressor responses to three incremental doses of intravenously infused noradrenaline (50, 100 and 150 ng min⁻¹ kg⁻¹) were obtained before and after thymoxamine to assess the degree of α-adrenoceptor blockage.

In the recently injured tetraplegic patient in spinal shock, two doses of saralasin (1 and 5 μg min⁻¹ kg⁻¹) were infused, each for 20 min. In the normal subjects, a similar protocol was used except that plasma noradrenaline levels were not measured and thymoxamine and noradrenaline were not administered.

Statistical analysis was performed with Student's t-tests and analysis of variance where appropriate. Means of the two baseline measurements taken 5 min apart were used as predrug levels, except in the case of plasma noradrenaline levels, when only one measurement was obtained. Results are expressed as means ± SEM. Mean blood pressure was calculated by adding one-third of pulse pressure to diastolic blood pressure.

Results

**Blood pressure and heart rate responses to saralasin**

In each tetraplegic patient there was an immediate pressor response to all three doses of saralasin. The maximum rise in blood pressure was from 132 ±14/65±4 to 168±15/85±5 (P<0.001 and <0.001), 141±8/72±3 to 175±9/91±4 (P<0.001 and <0.001) and 120±7/65±5 to 170±10/88±4 (P<0.001 and <0.001) mmHg during infusion of 0.5, 1.0 and 5.0 μg min⁻¹ kg⁻¹ respectively. The pressor response was not dose-dependent (Fig. 1). The maximum response usually occurred about the fifth minute of infusion, after which the blood pressure gradually
returned to the basal level (Fig. 2). A depressor response to intravenous saralasin occurred towards the end of the 20 min period of infusion in only one patient. In the tetraplegic patients there was a fall in heart rate which appeared to be in response to the elevation of blood pressure. During infusion of 0.5, 1.0 and 5.0 μg of saralasin min⁻¹ kg⁻¹, heart rate fell from 60 ± 3 to 51 ± 3 (P < 0.001), 62 ± 3 to 51 ± 4 (P < 0.001) and 67 ± 3 to 54 ± 2 (P < 0.002) beats/min respectively. In the recently injured tetraplegic patient in spinal shock, responses to the two doses of saralasin were similar in magnitude to the responses of the other patients (mean blood pressure rise of 22 and 21 mmHg and heart rate fall of 10 and 12 beats/min with 1 and 5 μg min⁻¹ kg⁻¹ respectively). In none of the tetraplegic patients was there a rebound increase in blood pressure after stopping infusion. In the normal subjects there were smaller changes in blood pressure and heart rate during the early period of saralasin infusion (Fig. 1). Blood pressure rose from 109 ± 1/62 ± 2 to 111 ± 3/69 ± 2 (not significant and P < 0.01), 109 ± 2/64 ± 2 to 115 ± 4/72 ± 4 (P < 0.05 and < 0.05) and 107 ± 2 to 109 ± 5/70 ± 4 (not significant and P < 0.05) mmHg and heart rate fell from 68 ± 3 to 61 ± 3 (P < 0.01), 64 ± 4 to 62 ± 5 (not significant) and 64 ± 3 to 60 ± 4 (P < 0.05) beats/min during infusion of 0.5, 1.0 and 5.0 μg of saralasin min⁻¹ kg⁻¹ respectively. No depressor responses occurred in the later periods of infusion.

Plasma noradrenaline, plasma renin activity and plasma aldosterone levels

Plasma noradrenaline levels were obtained in five tetraplegic patients. No change occurred 3 and 5 min after the start of saralasin infusion (Table 1), when the pressor response was usually maximal. There was no significant difference between the tetraplegic patients and normal subjects in their basal plasma renin levels, which spanned a wide range. The pressor response in the tetraplegic group, however, was higher, regardless of the prevailing level of plasma renin activity, and the mean blood pressure rise was above 15 mmHg in most

![Graph](image-url)

**FIG. 1.** Maximum change (Δ) in mean blood pressure and heart rate in tetraplegic patients (○) and normal subjects (controls, □) in the immediate period after each of three doses of saralasin infused intravenously. The bars indicate ± SEM.

![Graph](image-url)

**FIG. 2.** Intra-arterial blood pressure (BP) and heart rate (HR) in the tetraplegic patient in spinal shock before, during and after intravenous infusion of 1.0 μg of saralasin min⁻¹ kg⁻¹. Similar responses occurred in the chronically injured tetraplegic patients.
infusions, unlike the normal subjects (Fig. 3). Plasma renin activity did not rise either during (Table 2) or immediately after infusion except in one tetraplegic patient. During 1.0 and 5.0 μg of saralasin min⁻¹ kg⁻¹ infusions, his plasma renin level rose from 1.9 to 10.8 and from 2.9 to 11.6 pmol h⁻¹ ml⁻¹ respectively, while his blood pressure fell from 140/80 to 124/74 mmHg and from 114/76 to 108/72 mmHg respectively. Basal plasma aldosterone levels were similar in tetraplegic patients and in normal subjects and there were insignificant elevations in both groups after infusion (Table 3).

**Blood pressure and heart rate responses to saralasin after thymoxamine**

Infusion of the α-adrenoceptor blocker thymoxamine caused a fall in systolic and diastolic blood pressure from 145±11/75±3 mmHg to 124±11/67±3 mmHg after 30 min (P<0.01 for each). Heart rate rose from 66±5 to 80±5 beats/min (P<0.01). Infusion of saralasin (5 μg min⁻¹ kg⁻¹) 30 min after infusion of thymoxamine resulted in a similar pressor response but a greater fall in heart rate (Fig. 4). Blood pressure rose to 177±10/91±3 (P<0.001 and <0.001) mmHg and heart rate fell to 59±3 (P<0.002) beats/min. In six tetraplegic patients, noradrenaline was intravenously infused before and after thymoxamine. There was initially, as previously reported, an enhanced pressor response [10], which was significantly reduced to all three doses (P<0.05 for each) after thymoxamine (Fig. 5). The fall in heart rate was similar after thymoxamine, suggesting a greater response for a given rise in blood pressure.

**Discussion**

The intravenous administration of saralasin is often accompanied by an immediate transient pressor response in man [1, 2, 11, 12]. Because of the close structural relationship of saralasin with angiotensin II this effect has been ascribed to partial agonism, but it is not clear if this results from direct myotropism or an interaction with the sympathetic nervous system [13]. Evidence in favour of the latter mechanism relates to observations in normal and hypertensive patients, some on

### TABLE 1. Plasma noradrenaline levels (nmol/l ± SEM) in five tetraplegic patients before and 3 and 5 min after initiating infusion of different doses of saralasin

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Saralasin (μg min⁻¹ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>0</td>
<td>2.08±0.89</td>
</tr>
<tr>
<td>3</td>
<td>2.03±0.91</td>
</tr>
<tr>
<td>5</td>
<td>1.65±0.69</td>
</tr>
</tbody>
</table>

**Fig. 3.** Maximum change in mean blood pressure (Δ) in the early period after infusion of saralasin on the ordinate plotted against plasma renin activity on a log scale on the abscissa for tetraplegic patients (●) and controls (○). An arbitrary line has been drawn at the 15 mmHg mark. All but one of the points for the tetraplegic patients lie above this level and points for all the normal subjects lie below. The immediate pressor response to all doses of saralasin was enhanced in the tetraplegic patients regardless of basal plasma renin activity.

### TABLE 2. Plasma renin activities (pmol h⁻¹ ml⁻¹) ± SEM before and at the end of a 20 min infusion of different dose rates of saralasin

<table>
<thead>
<tr>
<th>Saralasin (μg min⁻¹ kg⁻¹)</th>
<th>Plasma renin activity (pmol h⁻¹ ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
</tr>
<tr>
<td>Tetraplegic</td>
<td>1.17±0.55</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>0.58±0.14</td>
</tr>
</tbody>
</table>

There were no significant differences after infusion.
**TABLE 3. Plasma aldosterone levels (pmol/l) ± SEM before, at the end (20 min) and 5 min after different dose infusions of saralasin**

There were no significant differences after infusion.

<table>
<thead>
<tr>
<th>Saralasin (µg min⁻¹ kg⁻¹)</th>
<th>Plasma aldosterone (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>215 ± 99</td>
</tr>
<tr>
<td>+5</td>
<td></td>
</tr>
<tr>
<td>Normal subjects</td>
<td>194 ± 39</td>
</tr>
</tbody>
</table>

**FIG. 4.** Histograms denoting maximum changes (△) in mean blood pressure and heart rate in tetraplegic patients during infusion of 5 µg of saralasin min⁻¹ kg⁻¹ before and after β-adrenergic blockade.

**FIG. 5.** Maximum changes (△) in mean blood pressure and heart rate in six tetraplegic patients during infusion of noradrenaline before and after administration of the α-adrenergic blocker thymoxamine.
haemodialysis, in whom saralasin infusion was accompanied by a rise in plasma noradrenaline but not plasma adrenaline levels [3, 14]. In the earlier study [3] the change in plasma noradrenaline correlated significantly with the change in blood pressure in the normal subjects but not in the patients on haemodialysis, and it was therefore concluded that the immediate pressor response to saralasin may also be mediated by an action on central or peripheral components of the autonomic nervous system. Others, however, found no change in levels of plasma noradrenaline and dopamine β-hydroxylase during infusion of saralasin [15]. Furthermore, the α-adrenoceptor blocker phentolamine did not attenuate the pressor response to saralasin in two hypertensive patients [2]. In our tetraplegic patients, all three doses of saralasin consistently produced a pressor response. In none of our patients was there evidence to indicate pathways between the cerebral and peripheral sympathetic nervous systems. Activation of central sympathetic pathways by saralasin, therefore, could not have participated in the rise in blood pressure.

In recently injured tetraplegic patients in spinal shock, it is difficult to activate the sympathetic nervous system, even at a spinal level [4]. After this phase, however, isolated spinal cord activity returns and sympathetic reflexes, which work independently of the brain, are evident. These can be readily elicited during stimulation of the urinary bladder or rectum, after which there is constriction of arteries and veins, an elevation in plasma noradrenaline levels and a marked rise in blood pressure [4, 8]. It is unlikely, however, that such spinal sympathetic reflexes were activated during infusion of saralasin as the levels of plasma noradrenaline were unchanged, even at the height of the pressor response. Furthermore, in the patient in spinal shock with absent spinal sympathetic reflexes, both doses of saralasin caused a pressor response which was similar in magnitude to the responses in the chronically injured tetraplegic patients.

In tetraplegic patients the vagus and glossopharyngeal nerves are intact and the afferent and efferent components of the baroreceptor reflex are functional. An elevation in blood pressure usually results in bradycardia because of increased cardiac vagal tone [10, 16]. During saralasin infusion, the reduction in heart rate closely followed the rise in blood pressure and was probably caused by increased vagal efferent activity, as has been observed during infusion of other vasopressor agents [10, 17].

Angiotensin II interacts peripherally with the sympathetic nervous system and may increase levels of noradrenaline in the synaptic cleft by a variety of mechanisms. This may result from increased biosynthesis [18], facilitation of release [19] or inhibition of re-uptake of noradrenaline [20]. Angiotensin II may also stimulate catecholamine release from the adrenal medulla [21]. To exclude similar actions of saralasin, resulting ultimately in activation of peripheral α-adrenoceptors, infusion was repeated after the α-adrenoceptor blocker thymoxamine [22–24]. Thymoxamine was chosen because of its relatively short half-life and because it does not appear to have the non-specific vasodilating effects of phentolamine [25], which can complicate the interpretation of results. To assess the degree of α-adrenergic blockade in our patients, the pressor responses to noradrenaline infusion were obtained before and after thymoxamine administration, after which there was a substantial shift of the response to the right. Re-infusion of saralasin after thymoxamine, however, caused no reduction in the pressor response, indicating that the pressor response was unlikely to be due to stimulation of post-synaptic α-adrenoceptors. Catecholamines released from the adrenal medulla could not have played a role as the pressor response should have been either attenuated or reversed by thymoxamine, depending on the amounts of noradrenaline or adrenaline released. The immediate pressor response to saralasin in these patients therefore appears to be the result of an angiotensin II-like myotropist effect.

Previous studies have indicated, particularly in hypertensive man, a relationship between the magnitude of the pressor response and the basal level of plasma renin. The suggestion is that with a lower basal renin level there are a larger number of unoccupied angiotensin II receptors and therefore a greater pressor response [2]. In neither our tetraplegic patients nor in the normal subjects was there a relationship between the magnitude of the pressor response and the basal level of plasma renin activity. Although the number of our subjects was small, there was a wide range of basal plasma renin activities. The pressor response to saralasin therefore appears to be determined by factors other than renin and angiotensin II levels and thus the degree of angiotensin II receptor occupancy. Tetraplegic patients have an enhanced pressor response to various vasoactive agents, including noradrenaline and angiotensin II [10, 17]. These responses do not appear to be the result of changes in receptor number or affinity [26] and are probably the result of disruption of the sympathetic efferent component of the baroreceptor reflex. It is tempting to speculate that in patients with low-renin hypertension, in whom an
enhanced pressor response to saralasin is attributed to low angiotensin II receptor occupancy, alternative mechanisms, possibly neural in origin [27], may account for the observed response.

Tetraplegic patients usually have either a normal or elevated basal level of plasma renin activity [28, 29] and it is not known to what extent this contributes to the maintenance of their blood pressure. Even at the end of a 20 min infusion, which should have resulted in sustained and adequate plasma levels of saralasin [30], there was little change in blood pressure, except in one patient. This does not exclude a role for angiotensin II in blood pressure control in these patients and may indicate that a longer period of infusion is necessary to overcome the initial partial agonist effects of the drug. Plasma renin activity in both the tetraplegic patients and the normal subjects showed minimal changes, except in one tetraplegic patient in whom the elevation in renin levels may be related to the fall in blood pressure. Saralasin caused an insignificant elevation in plasma aldosterone levels in both groups of subjects. These responses are similar to previously observed changes [11] and suggest an angiotensin II-like effect on the zona glomerulosa of the adrenal cortex.

Our studies therefore indicate that there is a consistent immediate transient pressor response to saralasin in tetraplegic man. These responses appear independent of central sympathetic facilitation and are unlikely to be due to peripheral sympathetic interactions, as plasma noradrenaline levels are unchanged and the responses are unaffected by α-adrenoceptor blockade with thymoxamine. The observed effects are therefore likely to be due to intrinsic angiotensin II-like activity on vascular smooth muscle. These partial agonist properties of saralasin are of significance in relation to both clinical and pharmacological studies designed to explore the contribution of angiotensin II to vascular tone and blood pressure control.

Acknowledgments

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