ANGIOTENSIN CONTROL OF RENIN RELEASE/Keeton et al. 531


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26. Gross R, Ruffmann K, Kirchheim H: The separate and combined effects

of common carotid occlusion and nonhypotensive hemorrhage on kidney

SUMMARY Circulating angiotensin II is said to inhibit renin

release by a direct, intrarenal action. This effect of angiotensin was

studied indirectly using the selective angiotensin II antagonist

saralasin (1-Sar-8-Ala-angiotensin II) in conscious normal, sodium-

depleted, and sodium-loaded rats. Saralasin caused a dose-related

increase in plasma renin concentration (PRC) in normal and

sodium-depleted rats, but had no effect on PRC in sodium-loaded

animals. However, saralasin was 300 times more active in sodium-
depleted rats than in normal rats. Saralasin caused hypotension and

tachycardia in sodium-depleted rats but did not alter the hypotensive

effect of saralasin in the latter. Saralasin potentiated phentolamine-

induced renin release, hypotension, and tachycardia in normal rats, and

this potentiated renin release was blocked by propranolol. We

A DECADE AGO, Vander and Geelhoed1 proposed a

pressure-independent mechanism by which circulating levels of

angiotensin II inhibited renin secretion through a direct

intrarenal action. In the ensuing years, support for their

hypothesis has been provided by other investigators, 6–9 and

these later studies have supported an intrarenal site of

action 10 which appears to be independent of sodium

metabolism. The use of specific angiotensin antagonists in

the study of this mechanism has been limited to one study

with the isolated perfused kidney, 9 and to several studies in

vivo 10,11 on animals exposed to anesthesia, an intervention

which alters renin release per se. 10,11

The selective angiotensin receptor blocking agent sarala-

sin (1-Sar-8-Ala-angiotensin II) has been shown to potenti-

ate the hypotensive action and renin release elicited by a

peripheral vasodilating agent. 12 In addition, saralasin itself

elevates serum renin activity 5-fold 13 in the absence of any

change in blood pressure or heart rate in rats 13 and in man. 14

Recent studies on vasodilator-treated, hypertensive patients

have demonstrated propranolol blockade of saralasin-

induced renin release. 15 These recent observations 12–15 and

the limitations, i.e., anesthesia and surgical intervention, of

the previous studies 10–11 prompted an examination of the in

vivo renin-releasing properties of saralasin in conscious rats

because, in the absence of anesthesia, this species is well

suited for studies of the renin-angiotensin-aldosterone

axis. 10–12,16–18 The characterization of saralasin-induced

The Effects of Altered Sodium Balance and

Adrenergic Blockade on Renin Release Induced in

Rats by Angiotensin Antagonism

T. KENT KEETON, PH. D., WILLIAM A. PETTINGER, M.D., AND

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conclude that a portion of saralasin-elicted renin release in sodium-
depleted rats is mediated by hypotensive activation of the carotid

baroreceptor reflex which increases sympathetic nervous activity in

the kidney. However, in sodium-depleted rats saralasin induced a

42-fold increase in PRC, whereas an equipotent hypotensive dose of

the vasodilator hydralazine caused only a 3.5-fold increase in PRC.

Thus, we find that saralasin appears to have a selective effect on renin

release over and above its hypotensive effect, which suggests an

angiotensin-mediated, feedback mechanism inhibitory to renin re-

lease. Thus, we have come to the conclusion that for part of sarala-

sin-induced renin release appears to be caused by disinhibition of

angiotensin suppression of renin secretion. This “short-loop” feed-

back mechanism is closely associated with intrarenal β-adrenergic

receptors, since propranolol impaired saralasin-induced renin re-

lease under all circumstances in our experiments.

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Methods

A total of 360 male Wistar rats weighing 250–350 g (Simonsen Laboratories) were housed in individual cages and exposed to light by an automated system from 6 a.m. to 6 p.m. The rats were given tap water and Purina rat chow containing 152 mEq of sodium per kg, ad libitum. Marked sodium depletion was achieved by providing a sodium-deficient diet (Nutritional Biochemical) containing 40 mEq of sodium per kg for 5 days and giving furosemide, intraperitoneally (ip), 10 mg/kg, during the first 3 days of dietary alteration. Sodium loading and volume expansion were achieved by administering deoxycorticosterone acetate (DOCA), 40 mg/kg, subcutaneously, and furosemide, subcutaneously (sc), in an olive oil emulsion and saline (0.9%) drinking water 2 days before the experiment.

Rats were decapitated and aortic blood was collected from the trunk as previously described.49 A single rat was killed for each determination of plasma renin concentration (PRC) and plasma saralasin concentration. Blood for plasma saralasin determinations was collected in isotonic ethylenediaminetetraacetate (EDTA) to prevent degradation of the peptide, and saralasin plasma levels were determined by radioimmunoassay as previously described.14

Drug dosage and time of administration are given in the figure legends. Saralasin was dissolved in 0.1 M tris(hydroxymethyl)aminomethane (Tris) HCl buffer, pH 7.5, which contained 0.2% (wt/vol) gelatin, and was administered subcutaneously. Propranolol, phentolamine, hydralazine, and furosemide were administered in 0.9% saline or 5% dextrose solution. The latter was used only in sodium-depleted rats. Propranolol and phentolamine were administered subcutaneously. Furosemide and hydralazine were administered intraperitoneally. Propranolol and phenolamine were dissolved by the addition of a few drops of glacial acetic acid and furosemide by several drops of 1 N NaOH. Control rats were given an equal volume of 0.9% saline or 5% dextrose by the corresponding route of administration.

Plasma renin activity (PRA) was measured by radioimmunoassay as previously reported,13 with several modifications.15 PRC was determined by modifying the PRA assay. The plasma to be assayed for PRC was diluted either 1:10 or 1:50 with nephrectomized (18 hours) rat plasma before the incubation step for the generation of A1, and the samples were assayed as usual. Saralasin concentrations up to 1,000 ng/ml did not interfere with the renin assay. As previously reported,14 A1 and angiotensin II (AII) do not interfere with the saralasin radioimmunoassay.

Direct mean arterial pressure (MAP) and heart rate in the unrestrained rat were obtained using an automatically cycling blood pressure device as described by Laffan and co-workers44 and Weeks41 with minor modifications. The system employed the use of a Narco RP-1500 pressure transducer, No. 7172 strain gauge coupler, and DMP-48 Physiograph. Direct blood pressure and heart rate were measured from an indwelling Weeks’ catheter22 placed in the abdominal aorta 5 days before the hemodynamic studies.

The unpaired Student’s t-test was used for all statistical analyses.

Results

Following the initial observation that saralasin (10 mg/kg, sc) could by itself cause a significant increase in circulating renin activity,15 this renin release and the kinetics of the concomitant disappearance of saralasin were characterized in normal sodium rats (Fig. 1). After subcutaneous injection (10 mg/kg) in Tris buffer, saralasin consistently caused a slight decrease in PRA at 10 minutes followed by a 5-fold increase at 20 minutes (Fig. 1). This elevation was relatively brief because PRA had returned to control levels by 40 minutes after injection, despite the fact that saralasin plasma levels were still 70 ng/ml. The approximate biochemical half-life of saralasin during this experiment was 8.3 minutes (Fig. 1). With the possible exception of a slight initial decrease, there was no change in MAP or heart rate after administration of saralasin (10 mg/kg) to normal rats (not shown). The initial increase in MAP and heart rate after saralasin may have been due to a modest intrinsic (angiotensin-like) activity of saralasin (see Discussion).

Since PRA and the plasma concentration of AII are known to vary inversely with sodium load,23 and since the...
The effect of alterations of net sodium balance on the ability of saralasin (10 mg/kg, sc) to increase renin release. Statistical P values are given in the middle of the line connecting the two groups compared. Each bar represents the mean ± SEM for six rats. DOC = deoxycorticosterone acetate.
administration of the same doses of saralasin used in constructing the dose-response curves in Figure 3. A significant decrease in MAP was observed with a dose of saralasin as low as 0.3 mg/kg. Further decrements in MAP down to a 15% decrease were observed with higher doses, but the 30 mg/kg dose had no more effect than the 10 mg/kg dose, indicating that the plateau portion of the dose-response curve that showed lowering of blood pressure had been reached at 10 mg/kg. Heart rate was unchanged at both time periods (10 and 20 minutes) with each dose given (Fig. 4).

Because several different stimuli to renin release are mediated via the sympathetic nervous system and can be blocked with a β-adrenergic antagonist,15-17, 25-29 and because propranolol can block saralasin-induced renin release in hypertensive man,19 propranolol was given before saralasin to both normal and sodium-depleted rats to determine whether saralasin-induced renin release in rats was affected by changes in adrenergic neurotransmission. In normal rats saralasin (10 mg/kg) caused a 10-fold increase in plasma renin concentration, and 97% of this increase was blocked by propranolol (1.5 mg/kg) (Fig. 5). This same dose of saralasin elicited a 92-fold increase in PRC in sodium-depleted rats, and pretreatment with propranolol mitigated 75% of this increment (this impairment appears somewhat less due to the distortion of linearity by the log scale used in Figure 5). The mean plasma saralasin concentration among the four groups treated with the drug was 172 ± 22 ng/ml (n = 24), and none of the groups differed significantly from the others with regard to this parameter.

Blood pressure did not change after propranolol and saralasin in normal rats (Table 1), and effective β-adrenergic receptor blockade was evidenced by a significant decrease in heart rate (Table 1). Furthermore, propranolol did not affect the ability of saralasin to lower blood pressure in sodium-depleted rats (Table 2). Saralasin (10 mg/kg) caused a 16% decrease in MAP in the absence of propranolol and a 14% decrease in MAP in the presence of propranolol. Unlike the data in Figure 4, saralasin-induced hypotension did result in significant tachycardia (Table 2). Propranolol pretreatment prevented the tachycardia and decreased the heart rate to below control levels (Table 2). Thus, propranolol could impair all of the saralasin-induced renin release in normal rats and the major part of it in sodium-depleted rats in the absence of any effect on the blood pressure response to saralasin alone.

Saralasin-induced renin release in sodium-depleted rats (Fig. 3) was associated with hypotension (Fig. 4 and Table 2) and tachycardia (Table 2). Since similar hemodynamic changes occurred in sodium-depleted rats 20 minutes after the administration of hydralazine (1 mg/kg), we compared the ability of hydralazine and saralasin to induce renin release in sodium-depleted rats. Hydralazine (1 mg/kg) caused a 25% increase in heart rate and a 21% decrease in blood pressure (not shown), whereas comparable values for saralasin (10 mg/kg) were a 20% increase in heart rate and a 16% decrease in blood pressure (Table 2). Despite the similar changes in heart rate and blood pressure elicited by the two drugs, hydralazine increased PRC only 3.5-fold as compared to the 42-fold increase seen after saralasin (Table 3). The plasma saralasin concentration was 221 ± 30 ng/ml (n = 6) in the saralasin-treated group.

Along the same lines, α-adrenergic blockade has been
shown to potentiate adrenergically mediated renin release. Likewise, phentolamine (10 mg/kg) and saralasin (10 mg/kg) elicited potentiated renin release in normal rats (Fig. 6). Saralasin, at a dose of 3 mg/kg, either did not increase renin (Fig. 3) or caused only a slight increase in renin (Fig. 6). However, phentolamine pretreatment resulted in a 114-fold increase in PRC with a dose of saralasin (3 mg/kg) which caused only a 1-fold increase in the absence of phentolamine (Fig. 6). α-Adrenergic blockade likewise potentiated the increase in PRC seen with the 30 mg/kg dose of saralasin; a 7-fold increase was seen in the absence of phentolamine and a 210-fold increase in the presence of phentolamine. Phentolamine alone caused a 7-fold increase in PRC; this was probably due to decreased blood pressure and possibly due to increased adrenergic transmission to the β-adrenergic receptors of the kidney which stimulate renin secretion. The plasma saralasin concentration of the two groups receiving the 3 mg/kg dose averaged 52 ± 6 ng/ml (n = 12). Comparable values for the 30 mg/kg dose were 948 ± 125 ng/ml (n = 12).

However, hemodynamic changes may have contributed to the potentiated renin release seen after phentolamine and saralasin. Phentolamine treatment resulted in a 32–34% decrease in MAP throughout the 30-minute time period monitored and a significant (P < 0.001) increase in heart rate (Table 4). Addition of 3 mg/kg of saralasin lowered the blood pressure further (43% decrease, P not significant from phentolamine alone) and significantly (P < 0.02) increased the tachycardia observed with phentolamine alone (Table 4). The administration of saralasin (30 mg/kg) after phentolamine resulted in a highly significant decrease (55%) in MAP as compared to phentolamine treatment, and again the tachycardia was significantly greater than that seen with phentolamine alone (Table 4). Even though the rats had an average blood pressure of 44 mm Hg, they remained calm and exhibited normal pressures and no apparent ill effects the next day.

Since the significant increase in heart rate (Table 4) after saralasin and phentolamine (as compared to phentolamine alone) was indicative of a general increase in sympathetic outflow, it was possible that the potentiated renin release seen after phentolamine and saralasin was mediated by β-adrenergic receptors (Fig. 6). This hypothesis was tested by pretreating normal rats with propranolol (1.5 mg/kg) prior to the administration of phentolamine (10 mg/kg) and saralasin (10 mg/kg) (Fig. 7). PRC increased significantly after saralasin (5-fold) or phentolamine (15-fold) alone, and the two drugs together elevated PRC more than 1,000 times the control value (Fig. 7). Propranolol alone had no effect on PRC, but blocked 73% of the renin release elicited by phentolamine (Fig. 7). Pretreatment with propranolol blocked 90% of the renin release caused by the phentolamine-saralasin drug combination (Fig. 7). The average plasma saralasin concentration for the three groups receiving saralasin was 238 ± 29 ng/ml (n = 18), and none of the groups differed significantly from the others in this regard.

### Discussion

The physiological control of renin release, which is a major rate-limiting step in A\textsubscript{11} formation, has been studied extensively but remains a perplexing problem. The complexity of the control of renin release is compounded by the fact that the ultimate product of renin, A\textsubscript{11}, has many potent physiological effects, yet renin release can be elicited by many different types of stimuli. Since this peptide possesses such powerful physiological activity, negative feedback control on renin release by plasma A\textsubscript{11} might ensure that formation of this autacoid would be modulated even in the

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**Table 1** Effect of Propranolol or Propranolol and Saralasin on Blood Pressure and Heart Rate in Normal Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (0)</td>
<td>10 min</td>
</tr>
<tr>
<td>Propranolol (1.5 mg/kg, sc)</td>
<td>99 ± 2</td>
<td>99 ± 2</td>
</tr>
<tr>
<td>Propranolol + saralasin (10 mg/kg, sc)</td>
<td>102 ± 3</td>
<td>104 ± 3</td>
</tr>
</tbody>
</table>

Propranolol was given at −10 minutes and saralasin or placebo injection was given at zero time. Each value is the mean ± SEM for six rats.

* P < 0.05; comparisons are between control values and treatment values within each group.

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**Table 2** Effect of Propranolol on Saralasin-Induced Hypotension and Tachycardia in Sodium-Depleted Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (0)</td>
<td>10 min</td>
</tr>
<tr>
<td>Saralasin (10 mg/kg, sc)</td>
<td>102 ± 3</td>
<td>90 ± 3*</td>
</tr>
<tr>
<td>Propranolol (1.5 mg/kg, sc) + saralasin</td>
<td>103 ± 2</td>
<td>95 ± 2*</td>
</tr>
</tbody>
</table>

Propranolol was given at −10 minutes and saralasin or placebo injection was given at zero time. Each value is the mean ± SEM for six rats.

* P < 0.05; comparisons are between control values and treatment values within each group.

†P < 0.01; comparisons are between control values and treatment values within each group.

‡P < 0.01; comparisons are between the two different treatment groups.
Saralasin to Induce Renin Release in Sodium-Depleted Rats

The rats were killed 20 minutes after drug administration. Each value is the mean + SEM for six rats.

* P < 0.001; comparisons are between the control and treatment values.

The initial observation that saralasin potentiated minoxidil-induced renin release in the conscious rat originally was thought to be due to the fact that angiotensin blockade potentiated vasodilatory drug hypotension in the rat. However, saralasin (10 mg/kg) by itself caused a 5-fold elevation of PRA without any change in blood pressure or heart rate. The study presented here (Fig. 1) confirms our original observations and indicates that saralasin may be able to block the “short-loop” control of renin release mediated by A11. These data imply that a certain part of renin secretion in the normal rat is modulated by circulating or locally generated (intrarenal) A11, or both. Steele and Lowenstein have reported similar data for the conscious rabbit. An infusion of saralasin (5 μg/kg per minute) caused an 8-fold increase in PRA with no change in MAP. In addition, direct infusion of saralasin (2 μg/kg per minute) into the renal artery of conscious, uninephrectomized dogs has been reported to increase PRA from 2.4 to 5.4 ng of A1/ml per hour with no change in MAP.

When saralasin has been infused into hypertensive patients there have been similar increments in PRA even though blood pressure or heart rate did not change. The inhibitory angiotensin system controlling renin secretion appears to be operative in humans, since systemically administered, suppressor doses of A11, have been reported to suppress renin release in normal humans and those with essential hypertension. Saralasin-induced increments in PRA in hypertensive humans in the absence of measurable hemodynamic changes appears comparable to the situation found in normal rats.

Conversely, it also appears that saralasin may, under certain circumstances, act temporarily as an agonist at the intrarenal angiotensin receptors inhibitory to renin secretion and thereby decrease renin release. An example of this apparent intrinsic activity is the decrease in PRA in sodium-depleted rats 10 minutes after saralasin (Fig. 2). In this case saralasin decreased PRA by 70% (Fig. 2) and MAP by 11.5% (Fig. 4) at 10 minutes. This is a departure from the normal reciprocal relationship between renin release and blood pressure. This inhibitory effect of saralasin on renin release in sodium-depleted rats is seen again in a more dramatic fashion in Figure 3. A very low dose of saralasin (0.03 mg/kg), resulting in a comparatively low plasma level (0.7 ± 0.1 ng/ml) of the drug, decreased PRC by 60% (Fig. 3) in the absence of any change in blood pressure (Fig. 4).

This marked decrease in plasma renin could be due to a direct agonist action of saralasin on the kidney angiotensin receptor which is inhibitory to renin release and/or on the kidney vasculature. In this respect, saralasin has been reported to act as a partial vascular agonist in conscious, normotensive dogs, and anesthetized normotensive dogs. Saralasin also can act as an agonist in vitro in the rat stomach and colon, and as an agonist in vivo in the rabbit renal vascular bed.

PRA (or PRC) and the circulating levels of A11 are known to vary inversely with net sodium balance, and the data in Figures 2 and 3 indicate that the ability of saralasin to elicit renin release also is inversely related to sodium balance. During sodium depletion, when blood volume is contracted and angiotensin levels are elevated, the inhibitory “short-loop” mechanism appears to be important in preventing hypersecretion of renin. Thus, sodium depletion amplifies the role of the “short-loop” mechanism in controlling renin release. In this situation saralasin elicits large increments in renin release.

Sodium-loaded, volume-expanded rats (treated with DOCA-saline) showed no measurable increase in renin release even at high doses of saralasin. This is an interesting finding since even low doses of saralasin have been reported to cause renal vasoconstriction in sodium-loaded rabbits. Renal vasoconstriction of the magnitude (50% in rabbits) reported by Mimran and his co-workers actually should decrease renin release via the intrarenal baroreceptor mechanism controlling renin release. Saralasin probably does act as a
pranolol was given 20 minutes before saralasin. Phentolamine was given 10 minutes before saralasin, and blood samples were collected 20 minutes after saralasin. All drugs were given subcutaneously. Statistical P values are given in the middle of the lines connecting the two groups compared. Each bar represents the mean ± SEM for six rats.

![Graph](http://circres.ahajournals.org/)

**FIGURE 7** Propranolol inhibition of the potentiated renin release caused by the saralasin-phenolamine drug combination. Prpranolol was given 20 minutes before saralasin. Phenolamine was given 10 minutes before saralasin, and blood samples were collected 20 minutes after saralasin. All drugs were given subcutaneously. Statistical P values are given in the middle of the lines connecting the two groups compared. Each bar represents the mean ± SEM for six rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (0) 10 min 20 min 30 min</th>
<th>Heart rate Control (0) 10 min 20 min 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prpranolol (10 mg/kg, sc)</td>
<td>97 ± 6 66 ± 3 65 ± 3 68 ± 4</td>
<td>340 ± 11 452 ± 10</td>
</tr>
<tr>
<td>Prpranolam + saralasin (3 mg/kg, sc)</td>
<td>104 ± 2 69 ± 3 56 ± 3 57 ± 3</td>
<td>352 ± 10 498 ± 7*</td>
</tr>
<tr>
<td>Prpranolam + saralasin (30 mg/kg, sc)</td>
<td>97 ± 2 71 ± 3 44 ± 3* 44 ± 2*</td>
<td>336 ± 13 519 ± 10*</td>
</tr>
</tbody>
</table>

Prpronanol was given at zero time and saralasin or placebo injection was given at 10 minutes. Each value is the mean ± SEM for six rats.

* P < 0.001; comparisons are between the saralasin treated groups and the response with phenolamine alone.

† P < 0.02; comparisons are between the two different saralasin doses after phenolamine.

Partial agonist in the rat renal vasculature under these circumstances, but the marked extracellular fluid volume expansion and increase in blood pressure attendant on sodium loading may have suppressed renin secretion to such an extent that further decrements were not possible.

Hemodynamic changes in sodium-depleted rats almost certainly contribute to saralasin-induced renin release. Angiotensin maintenance of arterial pressure in sodium-depleted states has been well established, and the lowest dose (0.3 mg/kg) of saralasin that caused an increase in PRC in sodium-depleted rats also caused a 7.1% decrease in MAP (Fig. 4). The maximum decrease (16.1%) in MAP occurred at a saralasin dose of 10.0 mg/kg (Fig. 4), but PRC continued to increase with the 30 mg/kg dose (Fig. 3). The largest dose (30 mg/kg) of saralasin caused only a 14.5% decrement in MAP (Fig. 4), yet PRC increased by 315-fold (Fig. 3). Others have noted the increased ability of saralasin to induce renin release after sodium deprivation. Given intravenously to conscious rabbits, saralasin 1 and 5 μg/kg per minute elicited a dose-related increase in PRA (up to a 10-fold increase), with a decrease in blood pressure occurring only at the higher dose. Differences in dietary sodium intake, basal renin values, and dose and route of administration of saralasin make it difficult to compare the data of Steele and Lowenstein with the data reported here.

After sodium depletion it is possible that saralasin causes greater hemodynamic changes within the kidney than it does in the general circulation. Renal blood flow decreased by 17% in rabbits deprived of sodium, with a proportionately larger decrease in outer cortical flow than inner cortical flow. Saralasin infusion restored renal blood flow to normal (with outer cortical flow increasing more than inner cortical flow) despite a 13-25% decrease in MAP. Even though restitution of renal blood flow may result, this increased flow would be associated with a decrease in perfusion pressure. Thus the hypotensive response to saralasin in sodium-depleted rats may contribute to renin release via the intrarenal baroreceptor mechanism.

Hemodynamic changes also must be considered in relation to the effects of β-adrenergic receptor blockade on saralasin-induced renin release. The prevention by propranolol of saralasin-elicited renin release in normal rats does not involve changes in MAP. Furthermore, hemodynamic changes cannot be evoked as an explanation for propranolol blockade of saralasin-induced renin release in sodium-depleted animals. Saralasin (10 mg/kg) was able to lower MAP in sodium-depleted rats to the same extent in the presence and absence of β-receptor antagonism (Table 2). The only difference between the two treatment groups in Table 2 is the mitigation by propranolol of the tachycardia caused by saralasin alone. However, saralasin-induced hypotension may cause a reflex increase in sympathetic discharge to the kidney, resulting in stimulation of juxtaglomerular cell β-receptors and thus in renin release. If this is true, then propranolol blockade of saralasin-induced renin release is similar to propranolol blockade of other types of sympathetically mediated renin release.

To test this hypothesis, we administered the systemic vasodilator hydralazine in a dose which caused hemodynamic changes in sodium-depleted rats similar to those seen...
after saralasin. Hydralazine has been shown to induce renin release by a β-adrenergic mechanism which can be blocked by propranolol. Despite the similar changes in heart rate and blood pressure elicited by the two drugs, hydralazine was a relatively poor stimulator of renin release as compared to saralasin (Table 3). Therefore, even though saralasin-induced renin release in sodium-depleted rats may in part involve activation of the baroreceptor and sympathetic nervous system mechanisms controlling renin release, other factors must be operative in order to precipitate such large increases in PRC. Blockade by saralasin of angiotensin-mediated suppression of renin secretion may be one of these factors.

The relationship between renin release and hemodynamic effects after combined treatment with phentolamine and saralasin may be even more complicated. Phentolamine alone caused a 7-fold increase in PRC (Fig. 6) associated with a 34% decrease in MAP (Table 4). The increment in angiotensin caused by the phentolamine-induced elevation in PRC appears to play a role in the maintenance of arterial pressure because saralasin treatment after phentolamine further decreased MAP (Table 4). Additional decrements in blood pressure alone cannot fully explain the synergism observed between saralasin and phentolamine in increasing PRC. At renal perfusion pressures below the autoregulatory capacity (70–80 mm Hg) of the dog kidney, renin release has been shown to remain constant despite further reductions in pressure. However, the rat kidney has been reported to stop autoregulation of blood flow at pressures below 95 mm Hg. Assuming that the control of renin release is the same in the rat and dog, the severe hypotension seen after phentolamine and saralasin (3 mg/kg) (MAP = 57 mm Hg, Table 4) should not have resulted in more renin release than with phentolamine alone (MAP = 68 mm Hg, Table 4), because both pressures are well below that necessary for autoregulation. However, PRC increased another 200-fold (Fig. 6).

Phentolamine has been demonstrated to produce renin release and β-adrenergic stimulation to the heart by reflex-mediated catecholamine release. Since the potentiated renin release seen after saralasin and phentolamine (Fig. 6) was associated with further increments in heart rate above the phentolamine-induced tachycardia (Table 4), intense β-adrenergic stimulation to the kidney may have contributed to their synergistic effect on renin release. Prevention by propranolol of 90% of the renin release caused by the phentolamine-saralasin drug combination is consistent with this speculation (Fig. 7).

Even though intrarenal α-adrenergic receptors which inhibit renin release may exist, the potentiated renin release seen here after phentolamine and saralasin can be attributed to synergism between the intrarenal baroreceptor and sympathetic nervous system mechanisms controlling renin secretion. In this respect, either renal nerve stimulation or isoproterenol infusion has been demonstrated to further increase renin release from dog kidneys at low perfusion pressures (50 mm Hg). This situation may be comparable to the potentiation by saralasin of vasodilator-induced renin release in normal rats.

Several previous studies were designed exclusively to study the effects of angiotensin receptor blockade on renin release.6 Bing gave the first clear demonstration of saralasin-induced renin release, but control plasma renin values were elevated (20–40 ng of AI/ml per hour), probably because of the use of a barbiturate anesthetic prior to blood sampling. The second report, by Oates and co-workers, involved the use of a barbiturate anesthetic, pentolinium and atropine, all of which have been shown to cause changes in renin release per se. Under these conditions, a 7-fold increase in PRA and PRC was noted, but this effect was seen only in the presence of ganglionic blockade by which itself caused a 35% decrease in blood pressure. Another angiotensin antagonist, 1-Sar-8-Gly-angiotensin II, has been shown to block the ability of angiotensin to inhibit renin secretion in anesthetized dogs. When given alone (1.0 μg/kg per minute), this inhibitor had no effect on renin release, but control PRA values (18 ng of AI/ml per hour) were greatly elevated in comparison to values found by others for conscious dogs (PRA < 1 ng of AI/ml per hour). Long-term treatment with the angiotensin antagonist 1-Sar-8-Ile-angiotensin II increased PRC 3-fold in renal hypertensive rats but had no effect on PRC in normal rats. Unfortunately, control PRC values were greatly elevated in both cases due to use of ether anesthesia which is known to cause renin release.

One final point to consider is the possible molecular mechanism of action of saralasin in eliciting renin release. There is evidence that β-adrenergic receptor stimulation increases intracellular levels of cAMP. There is evidence that angiotensin inhibition of adenylate cyclase was associated with a decrease in cAMP levels.50 Angiotensin also directly suppress renin release by inhibiting juxtaglomerular cell adenylate cyclase. Saralasin also directly inhibits renin secretion in vitro. Thus it appears that β-adrenergic stimulation results in increased levels of cAMP which in turn cause an increase in renin release by some unknown mechanism (probably involving calcium).

Angiotensin has been shown to inhibit adenylate cyclase activity in rat tail artery and to limit epinephrine stimulation of adenylate cyclase in rat uterus. In addition, angiotensin inhibition of adenylate cyclase was associated with a decrease in cAMP levels. Angiotensin also directly inhibited renin secretion in vitro. Thus, angiotensin may suppress renin release by inhibiting juxtaglomerular cell adenylate cyclase. Saralasin then may cause an increase in renin secretion by releasing juxtaglomerular cell adenylate cyclase from the inhibitory effect of angiotensin.

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