Saralasin-Induced Renin Release: Its Blockade by Prostaglandin Synthesis Inhibitors in the Conscious Rat

WILLIAM B. CAMPBELL, PH.D., EDWIN K. JACKSON, PH.D., AND ROBERT M. GRAHAM, M.B.

SUMMARY The angiotensin antagonist, saralasin, (10 and 30 mg/kg), increased serum renin activity (SRA) in normal, conscious rats from 2.7 ± 0.4 to 16.2 ± 3.7 and 22.5 ± 2.4 ng/ml/hr (p < 0.001), respectively, without markedly altering blood pressure or heart rate. Indomethacin, in a dose which inhibited the urinary excretion of prostaglandin E_{2} (PGE_{2}) by 75%, and arachidonate-induced hypotension by 83%, failed to alter basal SRA but inhibited saralasin-induced renin release by 99% and 87% at the 10 and 30 mg/kg doses, respectively. Indomethacin failed to alter basal hemodynamics or the hemodynamic response to saralasin. Propranolol (1.5 mg/kg) inhibited saralasin-induced renin release by 93% and enhanced the suppressant effect of indomethacin from 79% to 100%. Meclofenamate, another prostaglandin synthesis inhibitor, also blocked saralasin-induced renin release by 99% and 72% at the 10 and 30 mg/kg doses, respectively (p < 0.001). In sodium-depleted rats, saralasin (0.3 mg/kg) increased SRA from 12 ± 2 to 119 ± 6 ng/ml/hr (p < 0.001) and decreased blood pressure by 6% (p < 0.01). In these animals, indomethacin failed to alter basal SRA, but inhibited saralasin-induced renin release by 82%, urinary excretion of PGE_{2} by 79%, and arachidonate-induced hypotension by 81%

These findings suggest 1) that saralasin-induced renin release is mediated by renal prostaglandins, and 2) an interrelationship exists between the receptor controlling AIJ-mediated inhibition of renin release, which is blocked by saralasin, and the juxtaglomerular beta-adrenergic receptor. (Hypertension 1: 637-642, 1979)

KEY WORDS • indomethacin • meclofenamate • sodium depletion • propranolol • prostaglandin E_{2} • arachidonic acid • serum renin activity

ANGIOTENSIN II, the effector of the renin-angiotensin system, suppresses the release of renin by a negative feedback mechanism resulting from its direct action on the juxtaglomerular cells. Saralasin, an angiotensin antagonist, by blocking this negative feedback of angiotensin II, stimulates the release of renin in normal and sodium-depleted rats, dogs, rabbits and man. Since saralasin-induced renin release is inhibited by the beta-adrenergic blocking drug, propranolol, a beta-adrenergic mechanism appears to be involved in this form of renin release. Our previous studies indicate that sympathetically mediated renin release is inhibited by prostaglandin synthetase inhibitors. Thus, the present study was undertaken to determine the role of renal prostaglandins in saralasin-induced renin release by the use of these inhibitors.

Methods

Male Sprague-Dawley rats (250-300 g) were used in these studies (Simonsen Labs). The normal rats were maintained on a standard Purina Rat Chow diet containing 152 mEq Na/kg and 220 mEq K/kg and tap water ad libitum. In the studies involving sodium depletion, the rats were placed on a low-sodium diet (10 mEq Na/kg and 240 mEq K/kg, Nutritional Biochem) for 5 days, and given distilled water to drink. Additionally, on the first 2 days of dietary alteration, the animals were given furosemide, 10 mg/kg, I.P. All experiments were performed between 9:00 a.m. and 12:00 noon in conscious rats to

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eliminate the influences of diurnal variation, surgical stress, or anesthetic stress on the release of renin.

In the studies involving renin release, olive oil or a suspension of a prostaglandin synthetase inhibitor (indomethacin or meclofenamate, 5 mg/kg) in olive oil was injected subcutaneously at time zero. Ninety minutes later, saralasin or its vehicle (5% dextrose containing 0.2% bovine serum albumin) were administered subcutaneously, and the animals sacrificed at 110 minutes. In one group of rats, propranolol (1.5 mg/kg) or its vehicle (0.45% sodium chloride) was administered subcutaneously 80 minutes after the olive oil or indomethacin, and the protocol continued as described. Previous studies indicated that this dose of propranolol gave sustained plasma levels of 220 ± 19 ng/ml over a 40-minute period. The animals were sacrificed by decapitation, and the first 4 seconds of aortic blood (approximately 3 ml) collected in siliconized glass tubes on ice. The blood samples were allowed to clot, centrifuged, and serum separated at 4°C. The serum was then stored at −20°C until assayed for serum renin activity.

In the studies in which mean arterial pressure and heart rate were monitored, chronic Week's catheters were placed in the descending abdominal aorta below the origin of the renal arteries. After allowing a 2- to 5-day-recovery period, the conscious animals were placed in individual cages in a quiet room while mean arterial pressure and heart rate were monitored with a Narco RP-1500 pressure transducer, and recorded on a Grass model 7 Polygraph. After allowing a 1-hour stabilization period, a protocol identical to that described above was followed. In additional groups of normal and sodium-depleted rats with chronic Week's catheters in the descending abdominal aorta and jugular vein, indomethacin (5 mg/kg) or olive oil was injected subcutaneously at time 0. After 110 minutes, arachidonic acid (8 mg/kg) or its vehicle (0.1 M sodium carbonate) was injected intravenously via the jugular vein catheter, and changes in blood pressure were monitored.

Additional groups of normal and sodium-depleted rats were placed in metabolism cages to allow the collection of urine. After 2 days of equilibration, the urine was collected over an 8-hour period from 9:00 a.m. to 5:00 p.m. in rats injected at 9:00 a.m. with either olive oil, or indomethacin (5 mg/kg s.c.). The urine was then measured, filtered and stored frozen at −20°C until assayed for prostaglandins.

Serum renin activity was measured by the antibody trapping method of Poulsen and Jorgensen. Briefly, this method consisted of mixing at 4°C 25 μl of rat serum with 2 μl of a 3 M tris buffer containing 0.3 M EDTA, pH 7.2 and 3 μl of a 1:100 dilution of the anti-angiotensin I serum. This mixture was incubated at 37°C for 15–40 minutes, and returned to a 4°C ice bath. Then, 1 ml of cold 0.08 M barbital buffer, pH 8.6, containing 0.3% bovine serum albumin and 8000 counts per minute of [125I]-angiotensin I was added. After incubating overnight at 4°C, bound and free angiotensin I were separated with dextran-coated charcoal. Each serum sample was assayed in duplicate, and the results expressed as nanograms of angiotensin I generated at 37°C per milliliter of serum per hour (ng Al/ml/hr). Urinary prostaglandins were measured according to the method of Dray and coworkers involving acid lipid extraction, silicic acid column chromatography, and radioimmunoassay of prostaglandin E₁. The prostaglandin E₂ antibody was found to cross-react with PGE₂ by 14%, but cross-reacted less than 0.5% with PGA₂, PGB₂, PGD₂, 6-keto-PGF₁α, PGF₂α, and its 15-keto metabolites.

The drugs and their sources of supply are as follows: Saralasin (1-Sar-8-Ala-angiotensin II) (Norwich Pharmacol), arachidonic acid (Sigma), indomethacin (Sigma), meclofenamate (Parke Davis), propranolol (Sigma), prostaglandin E₂ (Upjohn Company), angiotensin I (Beckman), ³H-prostaglandin E₅ (New England Nuclear), and carrier free NaI²¹⁸ (New England Nuclear).

Statistical analysis were performed by using an unpaired Student's t test for single comparisons and an analysis of variance for multiple comparisons.

Results

Saralasin significantly increased SRA at both the 10 and 30 mg/kg doses (fig. 1). Indomethacin failed to alter the control renin levels but inhibited the saralasin-induced renin release by 99% (p < 0.001) at the 10 mg/kg dose and by 87% (p < 0.001) at the 30 mg/kg dose. Saralasin failed to alter blood pressure or heart rate at the time of measuring serum renin levels 20 minutes after injection (table 1). However, a transient 9% (p < 0.01) increase in heart rate was observed at 10 minutes after the administration of the 10 mg/kg dose and a 7% (p < 0.05) decrease in mean arterial pressure occurred, 30 minutes after its administration (data not shown). Similar hemodynamic effects were observed in the indomethacin pretreated rats receiving saralasin. No significant change in mean arterial pressure or heart rate was observed with the 30 mg/kg dose. When indomethacin was given alone, no change in mean arterial pressure or heart rate was observed.

Since propranolol also inhibited saralasin-induced renin release, the inhibitory effects of indomethacin and/or propranolol were examined on basal and saralasin-stimulated (30 mg/kg) renin release (fig. 2). Basal serum renin levels were inhibited 32% (p < 0.05) by indomethacin, 33% (p < 0.05) by propranolol, and 36% (p < 0.05) by the combination of indomethacin and propranolol. Saralasin-stimulated renin release was similarly inhibited with indomethacin, propranolol, and the combination of indomethacin and propranolol by 79%, 93%, and 100%, respectively (p < 0.001).

Meclofenamate, another prostaglandin synthesis inhibitor, reduced the basal serum renin levels by 21% (p > 0.1, NS) (fig. 3). Moreover, saralasin-induced renin release was inhibited by 99% (p < 0.001) and 72% (p < 0.001) at the 10 and 30 mg/kg doses, respectively.

When similar studies were performed in sodium-depleted rats, indomethacin failed to alter the elevated serum renin levels produced by sodium depletion (fig.
As in previous studies, sodium depletion potentiated the release of renin caused by saralasin with a 0.3 mg/kg dose elevating the serum renin activity from 12 ± 2 to 119 ± 6 ng/ml/hr (p < 0.001). Indomethacin inhibited saralasin-induced renin release by 82% (p < 0.001).

In sodium-depleted rats, saralasin (0.3 mg/kg) produced a slight (6%, p < 0.01) decrease in blood pressure 20 minutes after its administration while heart rate was unchanged (table 1). Indomethacin pretreatment resulted in a slightly lower heart rate and a slightly greater (10%) fall in blood pressure with saralasin.

Indomethacin reduced the urinary excretion of PGE$_2$ from 30.9 ± 3.4 to 7.8 ± 1.8 ng/8 hrs (p < 0.001) in normal rats and from 64.9 ± 15.3 to 13.8 ± 2.4 ng/8 hrs (p < 0.001) in sodium-depleted rats (table 2). This represented a 75% and 79% inhibition of the urinary excretion of PGE$_2$ in normal and sodium-depleted rats, respectively. In normal rats, administration of arachidonic acid (8 mg/kg, I.V.) resulted in a reduction in mean arterial pressure by 24.7 ± 6.1 mm Hg (p < 0.001). However, pretreatment with indomethacin inhibited the hypotensive effect of arachidonic acid by 83% (p < 0.001), as the mean arterial pressure only fell by 4.1 ± 1.4 mm Hg. In sodium-depleted rats, indomethacin also inhibited the arachidonate-induced hypotension by 81%.

**TABLE 1. Effect of Saralasin and/or Indomethacin on Heart Rate and Mean Arterial Pressure in Normal and Sodium-Depleted Conscious Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal rats</th>
<th>Sodium-Deplete rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102 ± 4</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Indomethacin 5 mg/kg</td>
<td>105 ± 3</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>Saralasin 10 mg/kg</td>
<td>99 ± 5</td>
<td>91 ± 4†</td>
</tr>
<tr>
<td>Indo + saralasin 10 mg/kg</td>
<td>100 ± 4</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Saralasin 30 mg/kg</td>
<td>99 ± 2</td>
<td>377 ± 9</td>
</tr>
<tr>
<td>Indo + saralasin 30 mg/kg</td>
<td>100 ± 2</td>
<td>357 ± 2</td>
</tr>
<tr>
<td></td>
<td>Mean arterial pressure (mm Hg)</td>
<td>Heart rate (beats/min)</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± sem for seven to nine rats measured 20 minutes after the injection of saline or saralasin.
†p < 0.05 compared with control.
FIGURE 2. Effect of indomethacin and/or propranolol on saralasin-induced renin release. Each value represents the mean ± SEM for eight rats. Statistical significance is indicated by the brackets above the compared bars. SRA = serum renin activity.

FIGURE 3. Effect of meclofenamate on saralasin-induced renin release. Each value represents the mean ± SEM for six rats. Statistical significance is indicated by the brackets above the compared bars. SRA = serum renin activity.
since sodium depletion amplifies the role of the angiotensin II inhibitory feedback in controlling renin release, the effect of prostaglandin synthesis inhibition was additionally examined in sodium-depleted rats. As in normal rats, indomethacin blocked saralasin-induced renin release in the sodium-depleted rats, without markedly altering the hemodynamic action of the drug. It should be mentioned that indomethacin was less effective in blocking saralasin-induced renin release at the high dose in normal rats and in sodium-depleted rats than in normal rats given the low dose. This may be due to the failure of indomethacin to totally block prostaglandin synthesis or due to saralasin stimulating renin release through a prostaglandin-independent mechanism.

In previous studies, we found that saralasin-induced renin release was inhibited by the beta-adrenergic antagonist, propranolol. The mechanism by which this inhibition occurred, however, was unclear. As saralasin-stimulated renin release without altering blood pressure or heart rate in normal animals, reflex activation of the sympathetic nervous system could not be involved in this form of renin release. Also, saralasin does not enhance the release of norepinephrine from the sympathetic neuron. Thus, it can only be concluded that there is a close functional relationship between the juxtaglomerular cell beta-receptor stimulatory effect on renin release, and the angiotensin II receptor inhibitory effect on renin release. However, the above observation with propranolol may explain the mechanism by which indomethacin suppresses saralasin-induced renin release, as our previous studies indicated that beta-adrenergically-mediated renin release could be blocked at the beta-receptor level by propranolol and at a site distal to the beta-receptor, by prostaglandin synthesis inhibitors. Consistent with this conclusion was the observation that the inhibitory effects of propranolol and indomethacin on saralasin-induced renin release were additive. Thus, indomethacin may be blocking saralasin-induced renin release by the same beta-adrenergic receptor-related mechanism as propranolol, but acting at a site distal to the beta-adrenergic receptor.

Alternatively, propranolol, indomethacin, and meclofenamate may have suppressed saralasin-induced renin release by a mechanism analogous to that observed previously with deoxycorticosterone and sodium chloride (DOC-NaCl). Treatment with DOC-NaCl suppressed basal renin release which reduced the circulating levels of angiotensin II, and thus the tonic inhibitory influence of the peptide on renin release. This contention was evidenced by the finding that saralasin failed to stimulate renin release with DOC-NaCl treated rats. Thus, propranolol and the prostaglandin synthesis inhibitors may have blocked saralasin-induced renin release by virtue of their ability to decrease basal renin levels, thereby reducing the tonic inhibitory influence of angiotensin II on renin release. Such a mechanism, however, appears unlikely since these drugs block saralasin-induced renin release but do not reduce basal renin levels.

Discussion

The present study was designed to examine the effect of prostaglandin synthesis inhibition, on the release of renin resulting from blockade of the angiotensin II-mediated negative feedback mechanism. In these studies, the release of renin, resulting from blockade of this mechanism by the angiotensin II antagonist saralasin was inhibited by two structurally-dissimilar prostaglandin synthesis inhibitors, indomethacin and meclofenamate. Also, since sodium depletion amplifies the role of the angiotensin II inhibitory feedback in controlling renin release, the effect of prostaglandin synthesis inhibition was additionally examined in sodium-depleted rats. As in normal rats, indomethacin blocked saralasin-induced renin release in the sodium-depleted rats, without markedly altering the hemodynamic action of the drug. It should be mentioned that indomethacin was less effective in blocking saralasin-induced renin release at the high dose in normal rats and in sodium-depleted rats than in normal rats given the low dose. This may be due to the failure of indomethacin to totally block prostaglandin synthesis or due to saralasin stimulating renin release through a prostaglandin-independent mechanism.

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This blockade of saralasin-induced renin release by propranolol and the prostaglandin synthesis inhibitors may constitute an important drug interaction. For example, Pettinger and Mitchell found that in hypertensive patients, the stimulation of renin release by saralasin outlasted the blockade of vascular angiotensin receptors by the drug. They proposed that this prolonged stimulation of renin release in the absence of vascular blockade caused the often observed rebound hypertension that follows saralasin withdrawal. If this is the case, the present study indicates that treatment with propranolol or a prostaglandin synthesis inhibitor would block the saralasin-induced renin release and would thereby prevent the rebound hypertension. Additionally, the present study demonstrates that while prostaglandin synthesis inhibitors block saralasin-induced renin release they do not alter the ability of saralasin to block vascular angiotensin receptors. Thus, it appears unlikely that prostaglandin synthesis inhibitors would interfere with a saralasin-infusion test for the diagnosis of angiotensin dependent hypertension.

The finding that both indomethacin and meclofenamate, two chemically dissimilar prostaglandin synthesis inhibitors, block saralasin-induced renin release, when administered in doses which decrease renal prostaglandin synthesis, suggests a role for prostaglandins in this form of renin release. Since angiotensin antagonists such as saralasin do not apparently stimulate renal prostaglandin release but rather inhibit angiotensin II-stimulated prostaglandin release, the mechanism of their involvement remains unclear. However, since the above studies were performed in isolated tissues and only measured the release of prostaglandin E-like material, it is possible that in vivo saralasin may increase the release of prostaglandins by an indirect mechanism possibly involving the sympathetic nervous system (see above). Alternatively, recent studies in anesthetized dogs indicate that PGI$_2$ is more potent than PGE$_2$ or PGD$_2$ in stimulating the release of renin. Also in rat in vitro renal cortical slices, PGI$_2$ increases the release of renin in a dose-related manner whereas PGE$_2$ is inactive. Thus, from the available information, PGI$_2$, rather than PGE$_2$ or PGD$_2$ appears to be the prostaglandin controlling renin release. If this is the case, saralasin may stimulate the release of renin by selectively increasing PGI$_2$ production without altering the production of PGE$_2$.

In summary, the prostaglandin synthesis inhibitors indomethacin and meclofenamate, in doses which inhibit the urinary excretion of PGE$_2$, block the increase in renin release caused by the angiotensin antagonist saralasin, independent of changes in systemic hemodynamics. This occurs in rats ingesting a normal sodium diet and in rats in which the renin releasing effects of saralasin are amplified by sodium depletion. Since a similar inhibitory effect is observed with the beta-adrenergic antagonist propranolol, it appears that saralasin-induced renin release may be associated with the beta-adrenergic receptor, and is possibly mediated by renal prostaglandins.

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