The Inhibitory Role of 12- and 15-Lipoxygenase Products on Renin Release

INDRA ANTONIPILLAI, JERRY L. NADLER, ELIZABETH C. ROBIN, AND RICHARD HORTON

SUMMARY Release of arachidonic acid from membrane phospholipids is a limiting step in the synthesis of both cyclooxygenase products and lipoxygenase products. The direct effects of prostacyclin and some lipoxygenase products on renin release were studied using rat renal cortical slices. Prostacyclin, at concentrations of $10^{-3}$ M, stimulated renin secretion, but this effect was short-lived. Leukotrienes or their precursor, 5-hydroperoxyeicosatetraenoic acid, did not affect basal renin release. In contrast, $10^{-6}$ M 12-hydroperoxyeicosatetraenoic acid and $10^{-6}$ M 12-hydroxyeicosatetraenoic acid were potent inhibitors of renin secretion. Similarly, 15-hydroperoxyeicosatetraenoic acid and its hydroxy derivative, 15-hydroxyeicosatetraenoic acid, at somewhat higher molar concentrations ($10^{-5}$ M) also reduced basal renin. These studies confirm prostacyclin as a potential renin secretagogue; however, its action in vitro is transient, probably because of its rapid degradation. Our studies provide new evidence that products of the 12-lipoxygenase and 15-lipoxygenase pathways, reported to be present in renal vascular tissue, are potent inhibitors of renin secretion and much more active on a molar basis on renin secretion than is prostacyclin. These studies suggest the potential presence of a dual system of stimulation and suppression that may regulate renin secretion in normal and clinical states. (Hypertension 10: 61-66, 1987)

KEY WORDS • renin • kidney • eicosatetraenoic acid • leukotrienes • prostacyclin

The juxtaglomerular apparatus involves both a major regulatory site, such as the afferent arteriole, and the juxtaglomerular cells, where renin is synthesized, stored, and released.1 Since these tissues are of vascular origin, it is not surprising that arachidonic acid (AA) products are produced and may play a role in the regulation of renin processing and secretion.

In response to various stimuli, certain membrane phospholipids can be rapidly hydrolyzed, leading to prostaglandin synthesis. Two pathways have been well defined for AA: cyclooxygenase (CO) and lipoxygenase (LO). The CO path leads to products such as prostacyclin (prostaglandin $I_2$) and thromboxane. LO activity leads to compounds such as the hydroperoxyeicosatetraenoic acids (HPETEs) and their respective hydroxyeicosatetraenoic acids (HETEs). The 5-LO enzyme found in leukocytes and monocytes yields the leukotrienes (LTs). Most literature on the LO family of compounds deals with their immune cell and inflammatory action, but these compounds are also potent smooth muscle constrictors.2,3 Vasodilator prostaglandins (prostaglandin $E_2$ and prostacyclin) have been shown to be potential renin secretagogues from the afferent arteriole that may act directly on juxtaglomerular cells.1 The present study confirms the direct effects on renin release of prostacyclin and also explores the effect of various LO products. We demonstrate that 12-eicosatetraenoic and 15-eicosatetraenoic acids are potent inhibitors of renin release and that a dual and local system regulating renin may exist in juxtaglomerular tissue.

Materials and Methods

Rat renal cortical slices were prepared by a method previously characterized.4,5 Briefly, male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 150 to 250 g were killed by decapitation. Kidneys were removed rapidly, freed of fat, and decapsulated. A Stadie-Riggs microtome (Arthur Thom-
as Co., Philadelphia, PA, USA) was used to obtain two slices (approximately 0.5 mm thick), from the superficial cortex of each kidney. The slices (weight, 15–30 mg) were placed in 25-ml flasks and washed with Krebs-Ringer bicarbonate with glucose (KRBG) buffer containing 0.01% bovine serum albumin. The washed slices were taken up into fresh KRBG containing (mM) 120 NaCl, 4.7 KCl, 1.2 MgSO4, 2.5 CaCl2, 1.2 KH2PO4, 26.8 NaHCO3, and 10 glucose at pH 7.4. The slices were preincubated for 15 minutes and then incubated in 2 ml of KRBG buffer at 37°C under 95% O2, 5% CO2 in a shaking water bath for five consecutive 15-minute intervals. Experiments were designed in such a way that each slice served as its own control; each slice was incubated for two 15-minute baseline periods, after which various agents were added and the response to an agent was observed for the next three 15-minute periods. The slices were exposed to agents for only 15 minutes, and the buffer was aspirated and replaced with fresh buffer during each subsequent 15-minute period. Baseline or control renin release was taken as the mean of two 15-minute intervals.

Prostacyclin sodium salt was obtained from Upjohn Diagnostic (Kalamazoo, MI, USA). LTBr, LTC4, and LTD4 were gifts from Merck, Frosst (Montreal, Quebec, Canada); 5-HP, 12-HP, 15-HP, and the HETEs were purchased from Biomol Research Laboratories (Philadelphia, PA, USA), and stored at −70°C. Pentex bovine serum albumin (fatty acid-free fraction V) was obtained from Miles Laboratories (Elkhart, IN, USA), and isoproterenol was obtained from Sigma Chemical Company (St. Louis, MO, USA). Prostacyclin was dissolved in 50 mM ice-cold Tris buffer, pH 9.3, whereas solutions of HETEs or HPETEs were prepared on ice just before use, according to a method described by Turk et al. The desired amount of HPETE (or HETE) was transferred to a 1-ml reaction vial (Pierce, Rockford, IL, USA), blown to dryness under nitrogen, and reconstituted in ethanol. The final concentration of ethanol in KRBG medium was 0.05%. This concentration of ethanol was also added to control incubations not exposed to test compounds and did not influence renin production. LTBr, LTC4, and LTD4 were diluted 1:10 with water, and final dilutions were made in KRBG buffer. Periodically, we tested the purity of the LTs, HPETEs, and HETEs by reverse-phase high performance liquid chromatography using a new gradient system that resolved prostaglandins, LTs, HPETEs, and HETEs in a single run. This system includes a Perkin-Elmer high performance liquid chromatograph (Norwalk, CT, USA) with an LC-85 ultraviolet detector and a 3 μm × 10-cm reverse-phase column. The gradient program and solvents (aqueous acetic acid/acetonitrile/methanol) are a modification of the method of Peters et al. Sensitivity of this method for LTs at 280 nm is 0.5 ng, while the sensitivity for 15-HETE, 12-HETE, 15-HPETE, and 12-HPETE is 2 ng at 235 nm. HPETEs (HETEs) were shown to be stable in the solution in the incubation medium for up to 3 hours under the conditions employed. Since HPETEs or HETEs are sensitive to light, dilutions of these compounds as well as experiments were conducted in the dark to prevent any major loss of potency.

Since viability was established by constancy in renin release and responsiveness to agonists as well as by low and unchanging release in the enzymes lactic dehydrogenase and glutamic oxaloacetic transaminase in control and HETE-treated samples over the entire period.

Renin release was quantitated as described previously. Briefly, 5 to 10 μl of the kidney slice incubate was added to 50 μl of renin substrate, prepared from nephrectomized rats. The substrate solution also contained 5 μl dimercaprol (2% solution wt/vol of benzyl benzoate; Sigma), 10 μl of 8-quinolinol sulfate (0.17 M), and 25 μl EDTA 2Na (4%) in a total volume of 300 μl Tris lysozyme buffer. The mixture was incubated for 75 minutes at 37°C in a shaking incubator. The angiotensin I thus generated was measured by the radioimmunoassay procedure of Haber et al. In all experiments, the agents used did not increase angiotensin I generation from substrate, nor was there a measurable effect of the agents on the radioimmunoassay.

All results are expressed as means ± SEM. Analysis of variance with both unpaired t tests and Duncan's multiple-range test was used to assess the significance of differences in renin release at different periods and with different concentrations. Statistical analysis was performed using the CLINFO computer system.

Results
Effects of Prostacyclin on Renin Release
In these experiments, 10−8 M prostacyclin stimulated renin release from cortical slices to 169 ± 6% of control levels at 15 minutes (p < 0.001), whereas neither 5 × 10−8 M prostacyclin (123 ± 4% of control levels; Figure 1) nor 10−9 M prostacyclin significantly altered renin release. The addition of vehicle for prostacyclin (10 μl of 50 mM Tris, pH 9.3) to control slices did not affect renin release. The stimulatory effect of prostacyclin on renin release did not persist longer than 15 minutes, and values fell to control levels during the 15- to 30-minute period.

Effects of 5-Lipoxygenase Products on Renin Release
LTBr, LTC4, and LTD4, at concentrations from 10−11 to 10−7 M, had no effect on renin. Similarly, their precursor, 5-HPETE (10−11-10−7 M), was also ineffective. Table 1 indicates a representative study of LTs and 5-HPETE at 10−7 M concentration.

Effects of 12-Lipoxygenase Products on Renin Release
Conversely, 12-HETE caused a marked inhibition of renin release in a dose-dependent manner (Figure 2). At 30 minutes, renin secretion by 10−10 M 12-HETE was similar to control levels. At a concentration of 10−8 M, 12-HETE slightly decreased renin release to 90 ± 7% of control levels, whereas 10−4 M 12-HETE reduced renin release to 82 ± 5% of basal val-
FIGURE 1. Effects of prostacyclin (PGI₂) on renin release from rat renal cortical slices. Baseline renin release was taken as a mean of −15 minutes and zero time. Effect of PGI₂ is expressed as a percentage of baseline following 15 (open bars) and 30 (hatched bars) minutes. Each value represents the mean ± SEM of five to 12 separate sets of experiments. Asterisk indicates significant difference (p<0.001) compared with control value at 15 minutes.

TABLE 1. Effect of Leukotrienes B₄, C₄, and D₄ and 5-Hydroperoxyeicosatetraenoic Acid on Renin Release

<table>
<thead>
<tr>
<th>Agents or vehicle added</th>
<th>Percent of control renin release (%)</th>
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<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Control</td>
<td>108±8</td>
</tr>
<tr>
<td>LTB₄ (10⁻⁷ M)</td>
<td>124±11</td>
</tr>
<tr>
<td>LTC₄ (10⁻⁷ M)</td>
<td>114±8</td>
</tr>
<tr>
<td>LTD₄ (10⁻⁷ M)</td>
<td>109±5</td>
</tr>
<tr>
<td>5-HPETE (10⁻⁷ M)</td>
<td>113±10</td>
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</table>

Incubations were performed as indicated in Methods. Values are means ± SEM, representing five to seven experiments in comparison to controls within a given period. LT = leukotriene; 5-HPETE = 5-hydroperoxyeicosatetraenoic acid.

FIGURE 2. Dose-related inhibitory effects of 12-hydroxyeicosatetraenoic acid (12-HETE) on percentage of renin release by rat renal cortical slices at 30 minutes. Values are means ± SEM, representing seven to 12 experiments. Single asterisk indicates that 12-HETE inhibited renin secretion significantly at 10⁻⁵ M compared with that of control or 10⁻¹⁰ M 12-HETE (p<0.02). Double asterisks indicate that larger doses (10⁻⁷ and 10⁻⁶ M) caused a higher inhibition (p<0.006) compared with that of control or 10⁻¹⁰ M 12-HETE.

TABLE 2. Renin Release in Control Slices and After Addition of 12-Hydroxyeicosatetraenoic Acid

<table>
<thead>
<tr>
<th>Control renin release (ng ANG I/mg tissue/hr)</th>
<th>12-HETE renin release (ng ANG I/mg tissue/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slice no. 0 min 30 min</td>
<td>Slice no. 0 min 30 min</td>
</tr>
<tr>
<td>1</td>
<td>8.9</td>
</tr>
<tr>
<td>2</td>
<td>9.9</td>
</tr>
<tr>
<td>3</td>
<td>23.8</td>
</tr>
<tr>
<td>4</td>
<td>6.5</td>
</tr>
<tr>
<td>5</td>
<td>9.9</td>
</tr>
<tr>
<td>6</td>
<td>18.6</td>
</tr>
<tr>
<td>7</td>
<td>27.7</td>
</tr>
<tr>
<td>8</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Mean ± SE 15.0±2.7 15.7±3.3 12.4±2 8.9±1.9*

ANG I = angiotensin I; 12-HETE = 12-hydroxyeicosatetraenoic acid.
*p<0.006, compared with respective control values.
of renin release exhibited considerable variation between incubations even when corrected for slice weight, as indicated previously by us, as well as by others, but values within the same slice did not greatly differ. This finding emphasizes the importance of using each slice as its own control.

Since 12-HETE is the metabolic reduction product of 12-HPETE, the effects of this unstable precursor on renin release were also examined. 12-HPETE was found to be a more potent inhibitor of renin secretion. Renin release induced by 10⁻¹¹ M 12-HPETE (15 minutes, 112 ± 6%; 30 minutes, 105 ± 9%; 45 minutes, 92 ± 5%) was similar to control levels. At 30 minutes, 10⁻¹⁰ M 12-HPETE slightly suppressed renin release to 87 ± 4% of basal values, and a highly significant inhibition (73 ± 7%) occurred at a concentration of 10⁻⁹ M (p<0.005), which was significantly different from values for control or 10⁻¹⁰ M 12-HPETE (Figure 3). A higher concentration of 10⁻⁸ M did not further inhibit renin secretion at 30 minutes (80 ± 5%).

In separate experiments, when slices were exposed to 10⁻⁹ M 12-HPETE for 15 minutes, there was a marked inhibition in renin secretion. When these same slices were washed twice with KRBG buffer (wash period) and then incubated, renin secretion returned to control levels during the next two 15-minute periods. When isoproterenol was added following a wash period, 12-HPETE-treated slices and isoproterenol-treated slices responded in a similar manner. With 10⁻⁶ M isoproterenol, renin release was stimulated at 30 minutes to 140 ± 10%, and at 45 minutes to 135 ± 9% of control values. Sequential addition of 12-HPETE and isoproterenol stimulated renin release to 135 ± 5% at 30 minutes and 138 ± 5% of basal values at 45 minutes.

Effects of 15-Lipoxygenase Products on Renin Release
15-HETE at a concentration of 10⁻⁶ M also decreased renin to 73 ± 7% of control values (p<0.01) at 30 minutes (Table 3). The threshold concentration that inhibited renin release by 15-HPETE was lower than that for 15-HETE: 10⁻⁷ M 15-HPETE suppressed renin secretion to 77 ± 6% of basal values (p<0.02).

Discussion
The present results address two issues. The first is whether prostacyclin mediates the release of renin, as indicated earlier. The second is whether LO products, also metabolites of AA, alter renin secretion. To our knowledge, no previous studies of LO products on renin secretion are available, although some reports indicate that LO products are closely involved in hormonal release in pancreatic islets and gonads. Under our experimental conditions, a relatively high concentration of prostacyclin (10⁻⁵ M) was necessary to induce a significant renin release (see Figure 1). The duration of this response lasted only 15 minutes. This finding is not unexpected as prostacyclin has a short half-life and is rapidly hydrolyzed to 6-keto-prostaglandin F₁α in aqueous solution. That this short action is due to rapid inactivation is suggested by data that iloprost, a stable analogue, induces renin release over a more prolonged period (unpublished observation, 1986). Our observations on prostacyclin are consistent with the reports by McGiff et al. as well as by Beierwaltes et al.

The important finding of the present study is that both 12-LO and 15-LO products are potent inhibitors of renin secretion by rat renal cortical slices in a dose-dependent manner. The 12-HPETE, 15-HPETE, 12-HETE, and 15-HETE that we employed differed in their efficacy to inhibit renin secretion. Whereas 12-HETE and 12-HPETE evoked significant renin inhibition at concentrations as low as 10⁻⁴ M, 15-HPETE and

![Figure 3. Time course inhibition of renin release by 12-hydroperoxycicosatetraenoic acid (12-HPETE). Each value represents mean ± SEM of eight to 12 experiments. Single (p<0.05) and double asterisks (p<0.005) indicate significant difference compared with values for control or 10⁻¹¹ M 12-HPETE, within a given period.](http://hyper.ahajournals.org/)

![Table 3. Effect of 15-Hydroxyeicosatetraenoic Acid and 15-Hydroperoxycicosatetraenoic Acid on Renin Secretion](http://hyper.ahajournals.org/)

<table>
<thead>
<tr>
<th>Agents or vehicles added</th>
<th>Percent of control renin release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Control</td>
<td>104 ± 6</td>
</tr>
<tr>
<td>15-HETE</td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>80 ± 5*</td>
</tr>
<tr>
<td>15-HPETE</td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>93 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SEM of five to seven experiments. 15-HETE = 15-hydroxyeicosatetraenoic acid; 15-HPETE = 15-hydroperoxycicosatetraenoic acid.

*p<0.02, **p<0.005, compared with respective control value.
15-HETE had to be given at concentrations of 10^{-7} M and 10^{-6} M, respectively. Local production as well as relative activity\textsuperscript{16} will determine which product might be potentially active in renin regulation. Another important finding was that both 12-HPETE and 15-HPETE appear to be more potent inhibitors of renin release than are their corresponding HETEs. Similar qualitative differences between HPETEs and the corresponding HETEs have been reported in other systems. For example, 12-HPETE was found to be more effective than 12-HETE in stimulating guanylate cyclase\textsuperscript{19} and modulating several human neutrophil functions, such as chemotaxis and chemokinesis.\textsuperscript{20} In pancreatic islet cells, 12-HPETE was able to stimulate insulin secretion, while 12-HETE was ineffective.\textsuperscript{13} In rat adrenocortical cells, 5-HPETE augmented adrenocorticotropic hormone–stimulated corticosteroid production while 5-HETE had no effect.\textsuperscript{21} Similarly, in the enhancement of histamine release in human neutrophils, 5-HPETE was reported to be more potent than 5-HETE.\textsuperscript{22} These differences among HPETEs and HETEs could be related to variations in the mechanisms of their actions on the secretion process or in the availability in the kidney cells of the response systems to different kinds of hydroperoxy or their corresponding hydroxy acids. The hydroperoxy derivatives are unstable and are rapidly reduced by cellular peroxidase to the more stable HETEs. Thus, HPETEs initially may be more potent and shorter-lived modulators, whereas the corresponding HETEs may be secondary, less potent, but longer-acting mediators.

We were unable to demonstrate any significant effects of exogenous 5-LO products on renin secretion. LT\textsubscript{B\textsubscript{4}}, LT\textsubscript{C\textsubscript{4}}, and 5-HPETE slightly increased renin secretion. Although LT-like products have been demonstrated in the perfusates of isolated kidneys challenged with a calcium ionophore,\textsuperscript{23} current evidence suggests that renal tissue does not form 5-HETE, and LT formation may be due to inflammatory cell infiltration.\textsuperscript{3, 24, 25}

The inhibition of renin release by HPETE (HETE) does not appear to be due merely to tissue damage or a nonspecific effect for several reasons. First, the renin secretion in slices exposed to 12-HPETE was reversed to control levels following a wash period. Second, the sequential addition of another renin secretagogue, such as isoproterenol, following HPETE action resulted in a similar stimulatory response as seen with isoproterenol alone. Third, the inhibitory effects were dose-dependent and acted at doses as low as picomolar levels. Fourth, the concentrations of the tissue enzymes glutamic oxaloacetic transaminase and lactic dehydrogenase, which usually increase when there is tissue damage, were not significantly different in the control and HPETE (HETE)-treated samples. Fifth, a related compound, 5-HPETE, which is a structural isomer of 12-HPETE and 15-HPETE differing only in the position of a single hydroperoxy group, failed to affect renin levels at doses varying from 10\textsuperscript{-11} to 10\textsuperscript{-6} M, indicating that not all HPETEs act similarly to inhibit renin secretion. This finding is consistent with the known structure-activity relationship of these compounds in other systems.\textsuperscript{26-28}

The 12-LO product 12-HETE is the most abundant arachidonate metabolite of lipoxygenase so far identified from rat glomeruli.\textsuperscript{18} Human glomeruli synthesize 12-HETE and 15-HETE in equivalent amounts.\textsuperscript{18} Similarly, blood vessels have also been shown to contain LO activity. For instance, rabbit and canine blood vessels can convert AA to 12-HETE,\textsuperscript{29, 30} whereas 15-HETE is the major LO metabolite of AA in human vessels.\textsuperscript{31} Since both prostacyclin and HETEs (HPETEs) can be synthesized by glomerular tissue (presumably by juxtaglomerular apparatus), as well as by vessels, our study suggests that a dual system of control may exist for renin regulation depending on the balance of CO and LO pathways for AA metabolism.

The mechanism of action of HETEs (HPETEs) on renin inhibition remains to be elucidated. It is well known that cyclic nucleotides (cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate) and calcium are two important second messenger systems in the renin secretory process, and although cyclic adenosine 3',5'-monophosphate is involved in the β-adrenergic stimulation of renin secretion,\textsuperscript{1} various data support the concept that Ca\textsuperscript{2+} is a key second messenger of renin release.\textsuperscript{1, 4, 32} The mechanism of action of 12-LO and 15-LO products in other systems for hormone release, smooth muscle cell migration, or mitogenic activity has been shown to be by Ca\textsuperscript{2+}-protein kinase C processes.\textsuperscript{33-35} Whether a similar mechanism is operative in juxtaglomerular cells for 12-HETE and 15-HETE action remains to be determined.

The physiological role of HETEs in renin secretion is not clear. AA stimulates renin secretion in vitro, and this effect is blocked by CO inhibitors. This sequence suggests that, under basal conditions, the CO pathway of AA is more active than the LO pathway. Recent studies suggest that angiotensin II acts through the calcium and the phosphatidylinositol pathway and that AA should be produced by these events.\textsuperscript{36, 37} We have preliminary evidence that one LO inhibitor prevents the renin-inhibiting action of angiotensin II. However, considerable work needs to be done before the LO pathway is proved to be involved in angiotensin action.

In disease states such as diabetes, HETE production in vessels is augmented while prostacyclin production is low, suggesting that HETEs may modulate endogenous vascular prostaglandin production\textsuperscript{38} and play a more prominent role in renin release. It has been proposed that prostaglandin deficiency may account for the defect of renin release in hyporeninemic hypoaldosteronism.\textsuperscript{38} The levels of HETEs in this syndrome are not known; however, increased glomerular production of HETEs in hyporeninemic hypoaldosteronism may reduce local prostacyclin formation and decrease renin levels. Therefore, the evidence presented in the present study that renin secretion is inhibited by 12-LO and 15-LO products suggests that, in certain states, LO-activated metabolites of AA may play an important role in the regulation of renin secretion.
Acknowledgments

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