12-Lipoxygenase Products Are Potent Inhibitors of Prostacyclin-Induced Renin Release (43083)

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Abstract. **In isolated human or rat glomeruli, arachidonic acid can be metabolized by the cyclooxygenase pathway to prostaglandins or by the lipoxygenase pathway to hydroxyeicosatetraenoic acids (HETES). We have recently shown that 12-lipoxygenase products are potent inhibitors of renin release. Since prostacyclin (PGI_2) is a potential renin secretagogue, we studied the direct effects of 12-lipoxygenase products on prostacyclin-induced renin secretion. Treatment of rat renal cortical slices with picomolar concentrations of 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and 12-HETE blocked the prostacyclin- or iloprost (an analog of PGI_2)-induced renin secretion. The inhibitory effects of 12-lipoxygenase products were not exhibited by the 5-lipoxygenase-derived products, leukotriene B_4 and 5-HPETE. These results suggest that HETES are not only potent modulators of prostacyclin actions on renin, but that the concerted actions of these compounds in cells may be critical determinants of the juxtaglomerular cell secretion of renin.** [P.S.E.B.M. 1990, Vol 194]

Prostaglandins (PG) made by renal vascular tissues appear to play an important role in renin release as blockade of PG systems has been shown to alter renin release both *in vivo* and *in vitro* (1). In kidney and vessels, arachidonic acid (AA) is also the substrate for another group of enzymes, the lipoxygenases (LO), that convert AA into different 12-hydroperoxyeicosatetraenoic acids (HPETES) which are reduced further by a glutathione-dependent peroxidase to the corresponding hydroxyeicosatetraenoic acids (HETES) (2–4). Recent studies have indicated that 12-HETE, an LO product from AA, is produced not only by platelets (5), macrophages (6), pancreatic islets (7), smooth muscle cells (8), adrenals (9), and vessels (3), but also by human, pig, and rat renal cortical tissue (10, 11). We have recently shown that 12-LO products, such as 12-HPETE and 12-HETE, are potent inhibitors of renin release (12). However, the role of LO products on prostacyclin (PGI_2) stimulation of renin is unknown. In this article, we report that 12-HPETE and its 12-hydroxy derivative are potent inhibitors of PGI_2-induced renin release.

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Materials and Methods

Rat (male, Sprague-Dawley, weighing 150–250 g, 42–54 days old) renal cortical slices were used for both static incubations and perifusion experiments (Endotronics Acusyst S perifusion system, Endotronics, Marietta, OH) by a method described previously (12–14). Briefly, using a Stadie-Riggs microtome, two slices (approximately 0.5-mm thick) were obtained from the superficial cortex of each kidney. For static incubations, slices (15–30 mg) were suspended in 2 ml of Krebs-Ringer bicarbonate with glucose (KRBG) buffer containing 0.01% bovine serum albumin, equilibrated with 95% O_2-5% CO_2, and incubated at 37°C for five consecutive 15-min periods. Each slice was incubated for two 15-min baseline periods, after which agents were added and the response to an agent was observed for the next three 15-min periods. The slices were exposed to fresh agents for 15 min and the buffer was aspirated and replaced with fresh buffer during each subsequent 15-min period. Baseline or control renin release was taken as mean of the first two 15-min periods. The standard KRBG medium contained (in millimolar concentrations) NaCl, 120; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 24.8; and glucose, 10 at pH 7.4.

For perifusions, two slices (75–125 mg of renal cortical tissue) were placed in each of six temperature-

controlled perifusion chambers and perifused with the standard KRBG medium as described previously (14). Slices were perifused for at least 60 min to stabilize before 10-min fractions were collected. After a 30-min baseline sampling, the agents were dissolved in 20 ml of KRBG buffer and perifused over a 20-min period. This was followed by a control KRBG buffer for a period of 50 min.

Iloprost, a stable analog of PGI_2 (ZK36,374), was a gift from Berlex Laboratories, Inc. (Cedar Knolls, NJ). PGI_2 (Upjohn Diagnostic, Kalamazoo, MI) was made up in ice-cold Tris (pH 9.3) buffer (50 m*M*), and solutions of HPETES, HETES, and leukotriene B_4 (LTB_4) (Biomol Research Laboratory, Philadelphia, PA) were dissolved in ethanol and prepared on ice just before use (12). 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. Since HPETES or HETES are sensitive to light, dilutions of these compounds as well as experiments were conducted in the dark to avoid any significant loss of potency.

Renin activity in the supernatant of the incubation or perifusion medium was measured by radioimmunoassay after angiotensin I generation according to the method of Haber *et al.* as described previously by us (12–14).

Data Analysis

The results are expressed as the mean ± SE percentage of control renin release. In the static incubation model, renin release during 15-, 30-, and 45-min periods was expressed as the percentage of baseline (i.e., mean of −15 min and 0 time) in the same slice. In the perifusion experiments, renin release during each collection period was expressed as the percentage of basal renin release in the same chamber during the first collection period (0–10 min). Statistical significance was determined with analysis of variance and a multiple comparison method (using either Duncan's or Dunnett's test, wherever appropriate).

Results

Effects of PGI_2 and Its Stable Analog Iloprost on Renin Release. Renin release from individual cortical slices was relatively stable during the entire 75-min incubation period. In a representative experiment, the mean renin release during −15 min and 0 time was 22.5 ng of angiotensin I/ng/hr (100%), at 15 min, 23.9 ng of angiotensin I/ng/hr (106%), at 30 min, 22.7 ng of angiotension I/ng/hr (101%), and at 45 min, 21.4 ng of angiotensin I/ng/hr (95%). The absolute levels of renin exhibit considerable variations between incubations even when corrected for slice weight as indicated previously by us (13) as well as by others (15), but values within the same slice do not greatly differ. This finding emphasizes the importance of using each slice as its own control. In a static incubation system, we compared the renin stimulatory response of PGI_2 with that of iloprost (Table I). PGI_2 (10^{-5} *M*) significantly stimulated renin secretion at 15 min to 169 ± 6% compared with control slices, 106 ± 5%, $P < 0.01$; however, the values at 30 min and 45 min fell almost to control levels. In contrast, iloprost (10^{-6} *M*) produced a similar increase in renin release which continued for 30 min (15 min 152 ± 5%, 30 min 155 ± 8%, both $P < 0.01$).

Effects of 12-LO Products on Iloprost or PGI_2-Induced Renin Release in Static Incubations. 12-HPETE and 12-HETE had an inhibitory effect on iloprost-induced renin secretion. The values at 15, 30, and 45 min of incubations are shown in Figure 1. Concentrations of 12-HPETE (10^{-11} *M*) which did not alter basal renin release significantly blocked iloprost (10^{-6} *M*)-stimulated renin secretion at both 15 and 30 min. The concentration of 12-HPETE (10^{-9} *M*) which did decrease basal renin release also reduced iloprost-induced renin secretion at all three time periods (Fig. 1). However, these values were not significantly different from iloprost + 12-HPETE at 10^{-11} *M*.

Similarly, 12-HETE (a metabolic reduction product of 12-HPETE) at 10^{-10} *M* concentration had no effect on basal renin secretion whereas 10^{-8} *M* concentration reduced basal renin release significantly at 30 and 45 min (Fig. 2). However, 10^{-10} *M* 12-HETE reduced iloprost-induced renin secretion slightly and 10^{-8} *M* 12-HETE significantly decreased iloprost action at all three time periods, i.e., 15, 30, and 45 min (Fig. 2).

In addition, both 12-HPETE and 12-HETE also blocked PGI_2-stimulated renin secretion (PGI_2, 168 ± 5%; PGI_2 + 12-HPETE (10^{-9} *M*), 94 ± 12%; PGI_2 + 12-HETE (10^{-8} *M*), 117 ± 7%, both $P < 0.05$ versus PGI_2.

Effects of 5-Lipoxygenase Products on Iloprost-Induced Renin Release. LTB_4 and 5-HPETE at 10^{-8} *M* and 10^{-7} *M* had no effect on basal or iloprost-induced renin secretion. Table II indicates a represent-

Table I. Effects of PGI_2 and its Stable Analog Iloprost on Renin Release[a]

Agents or vehicle added	% control renin release		
	15 min	30 min	45 min
Control	106 ± 5	101 ± 7	100 ± 7
PGI_2 (10^{-5} *M*)	169 ± 6[b]	118 ± 5	109 ± 16
Iloprost (10^{-6} *M*)	152 ± 5[b]	155 ± 8[b]	125 ± 12

[a] Incubations were performed as indicated in Materials and Methods. Values are mean ± SE representing five to eight experiments in comparison to controls within a given period.
[b] $P < 0.01$ versus control.

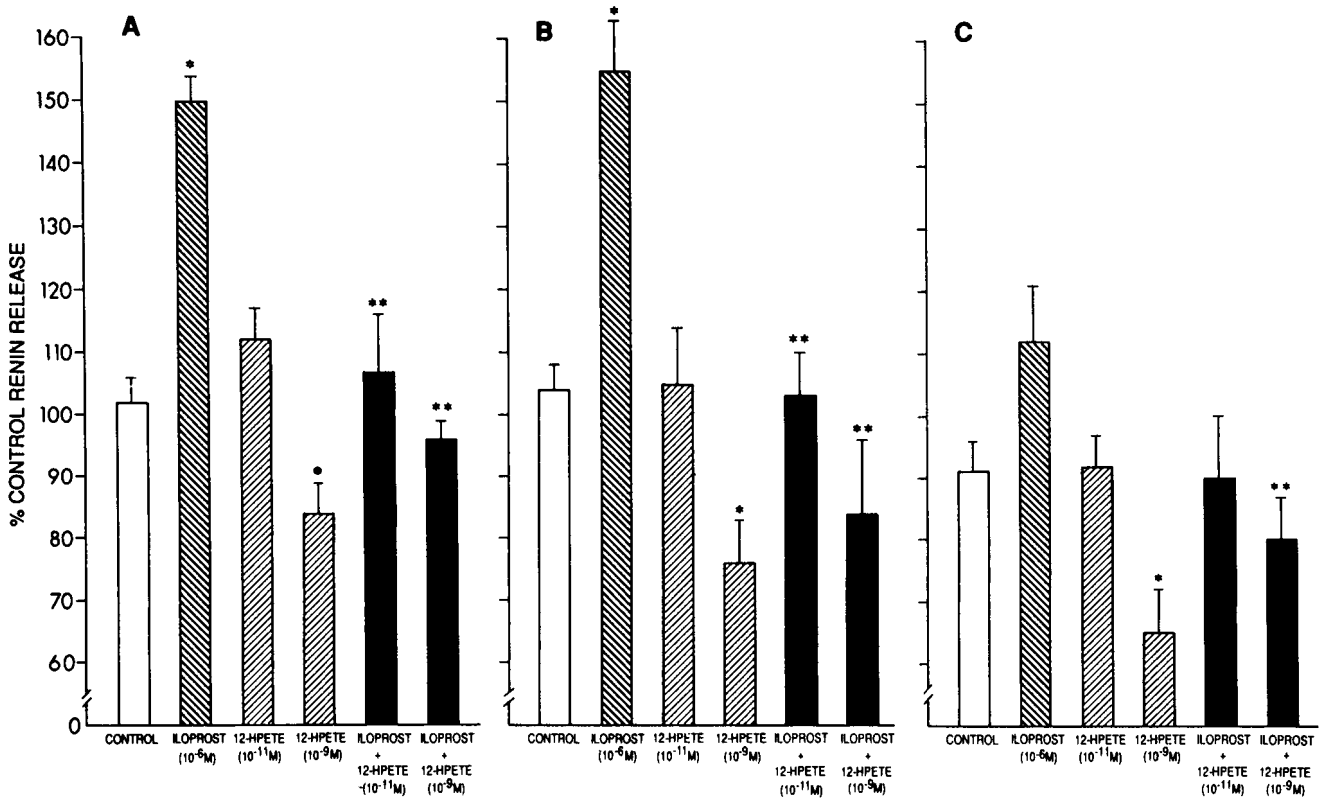

Figure 1. Effects of iloprost and 12-HPETE, separately or in combination, on percentage of renin release by rat renal cortical slices. Baseline renin release is plotted as a percentage of the mean of −15 min and 0 time values; effect of iloprost and 12-HPETE is expressed as a percentage of the baseline after 15 (A), 30 (B), and 45 min (C). Values are the mean ± SE representing five to seven experiments. * $P < 0.05$ versus control, ** $P < 0.05$ versus iloprost.

ative study of LTB_4 and 5-HPETE at 10^{-7} M concentrations.

Effects of 12-HPETE on Iloprost Effects in the Perifusion System. To fully evaluate the role of 12-HPETE and its time course on PG action, perifusion studies were performed. As indicated in Figure 3, control slices released renin in a relatively stable manner over the entire study period (100 min). Iloprost (10^{-6} M) caused a rapid increase in renin release. The values peaked at 40–50 min (i.e., 10 min after exposure of slices to this agonist) and returned to basal levels during a 60- to 70-min time period. The effects of 12-HPETE in perifusion are shown in Figure 4. 12-HPETE inhibited renin secretion after a 20-min lag period. The maximal inhibition was seen at the 60- to 70-min time period. When slices were treated with 12-HPETE (10^{-9} M) + iloprost, the peak renin response to iloprost was not only blocked (40–60 min) following its addition, but renin release was reduced below control values, somewhat similar to that seen with 12-HPETE alone (at 70 min). The renin levels then returned to basal control levels by 80–100 min (Fig. 3).

Effects of 12-HPETE on Isoproterenol (ISOP)-Induced Renin Release. To determine whether 12-HPETE also has effects on other mediators of renin secretion, the effects of ISOP, a β-adrenergic agonist, were assessed. ISOP (10^{-6} M) produced a significant and sustained increase in renin release in the perifusion set up (Fig. 5). Its effect was significant by 10 min after its addition, and renin release reached a plateau from 40 to 100 min. When 12-HPETE and ISOP were added to the perifusate, renin levels decreased significantly and values obtained were similar to those of control slices after 20 min ($P < 0.05$ versus control). There appeared to be a 15- to 20-min delay in the effect of 12-HPETE on ISOP-induced renin release.

Discussion

12-HETE is a major arachidonate LO product in both isolated human and rat glomeruli (10). The results of the present studies demonstrate for the first time that nanomolar concentrations of 12-HPETE and 12-HETE significantly decrease the PGI_2-induced release of renin. Since PGI_2 has a short half-life and is unstable in solution, studies were also carried out with iloprost (16). Picomolar concentrations of both 12-HPETE and 12-HETE also inhibited the action of iloprost or renin release. When compared with 12-HETE, 12-HPETE was more potent in our system. These quantitative differences between HPETES and HETES exist in a number of cell types (12).

Additional support for the potent inhibitory role of

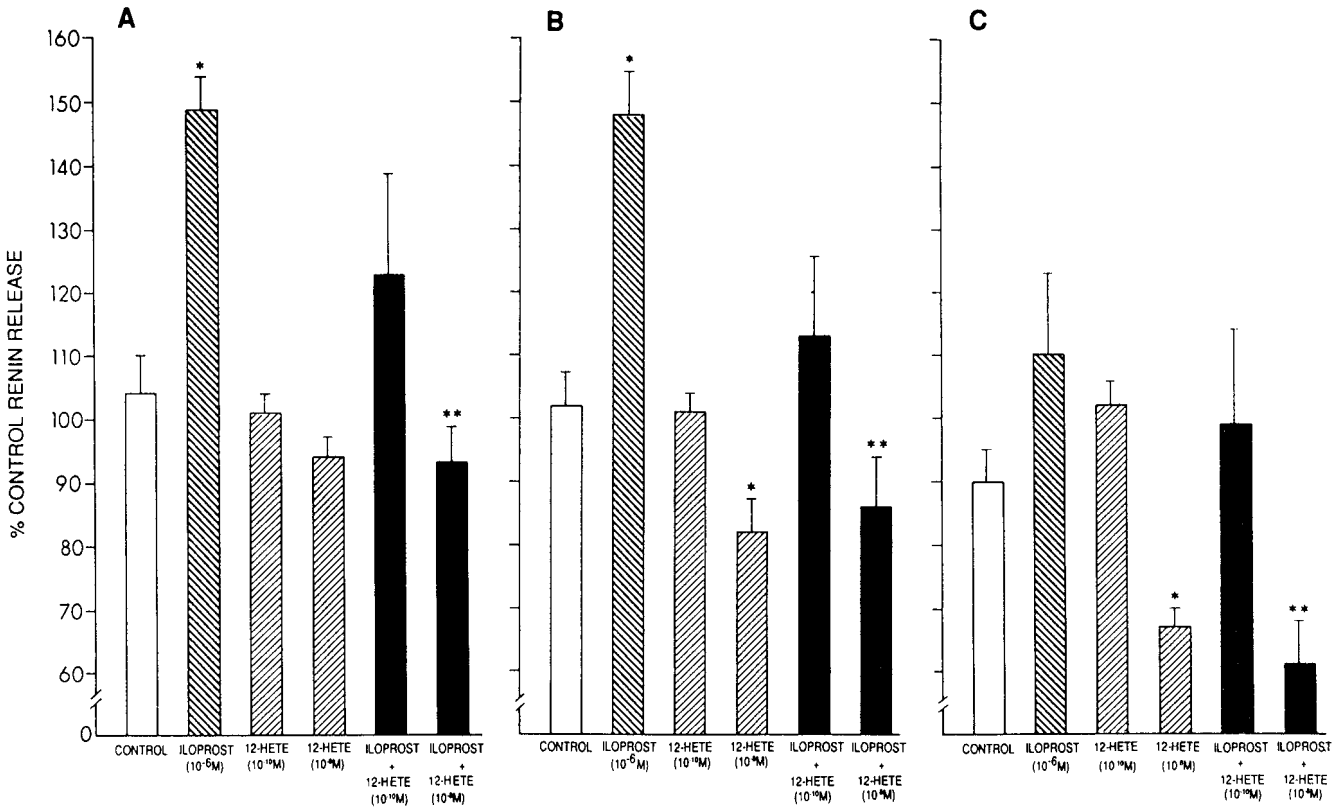

Figure 2. Effects of iloprost and 12-HETE, separately or in combination, on percentage of renin release by rat renal cortical. Baseline renin release is plotted as a percentage of the mean of −15 min and 0 time values; effect of iloprost and 12-HETE is expressed as a percentage of the baseline after 15 (A), 30 (B), and 45 min (C). Values are mean ± SE representing six to seven experiments. * $P < 0.05$ versus control, ** $P < 0.05$ versus iloprost.

Table II. Effects of LTB_4 and 5-HPETE on Renin Release[a]

Agents or vehicle added	% control renin release		
	15 min	30 min	45 min
Control	106 ± 3	93 ± 3	92 ± 6
LTB_4 (10^{-7} *M*)	122 ± 10	116 ± 18	104 ± 19
5-HPETE (10^{-7} *M*)	116 ± 9	119 ± 9	111 ± 7
Iloprost (10^{-6} *M*)	136 ± 7[b]	149 ± 9[b]	122 ± 13
Iloprost + LTB_4	133 ± 3[b]	160 ± 3[b]	121 ± 8
Iloprost + 5-HPETE	140 ± 12[b]	177 ± 14[b]	121 ± 9

[a] Incubations were performed as indicated in Materials and Methods. Values are mean ± SE representing five to seven experiments in comparison to controls within a given period.
[b] $P < 0.05$ versus control.

12-LO products on PG-induced renin was provided from the perifusion experiments, which are considered more physiologic since products from the renal cortical slices are constantly washed out and the contents of the medium are kept constant. Using this system, 12-HPETE blocked the iloprost action, during the 30- to 60-min time period and values dropped below the control slices at the 70-min time period (Fig. 3). The inhibitory action of 12-HPETE was also seen in ISOP-induced renin release; however, since the ISOP action on renin was prolonged, 12-HPETE reduced the renin release to basal levels. These studies suggest that 12-HPETE blocks both the short-lived stimulation by iloprost and the sustained increases by ISOP.

In a number of systems, LO products have been shown to reduce PG formation by inhibiting cyclooxygenase (CO) (17–19). In pig and bovine aortic endothelial cells, 12- and 15-HPETE inhibit PGI_2 synthesis (17, 18). The same has been reported for 12- and 15-HETES which are competitive inhibitors of vascular CO (19). Recent studies indicate that MDCK cells (derived from renal tubular epithelium), incubated with 12-HETE, have a reduced capacity to produce PGE_2 (20). These studies suggest that HETES have the capacity to decrease PG production. The data presented here indicate that HETES can also block the effect of added PGI_2 on renin release.

The inhibitory actions of 12-HPETE (HETE) on PGI_2 or ISOP do not appear to be simply toxic or due to a cellular damage for several reasons. First, the inhibitory effects were seen at concentrations as low as 10^{-11} *M*. Second, renin secretion in slices exposed to 12-HPETE alone was reversed to baseline levels following a wash period (12). Third, a related compound, 5-HPETE, which is structural isomer of 12-HPETE differing only in the position of a single hydroperoxy

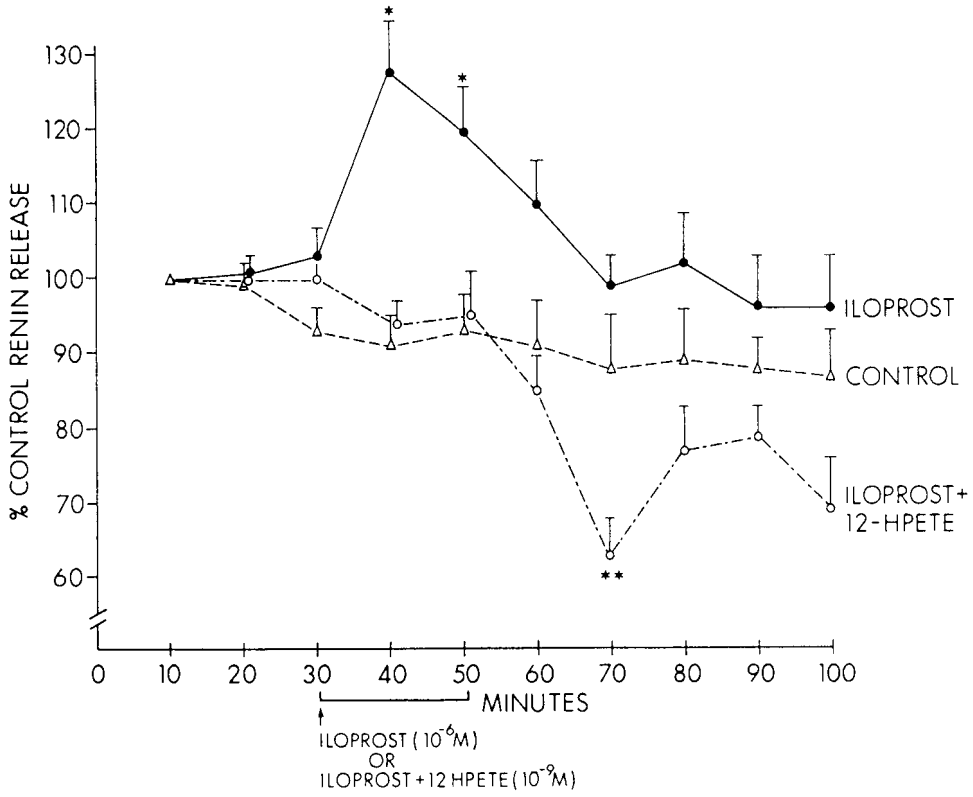

Figure 3. Effects of iloprost and iloprost + 12-HPETE on renin release by perifused rat renal cortical slices. Perifusions were performed as described in the text. The results are expressed as percentage of control renin release. Values represent mean ± SE of six to eight separate experiments. Iloprost significantly stimulated renin release at 40–50 min compared with control (*$P < 0.05$), while renin release with iloprost + 12-HPETE treatment was not significantly different from that of control. Iloprost + 12-HPETE significantly inhibited renin compared with control at 70 min (** $P < 0.05$). No significant differences were found between control and iloprost at 70 min.

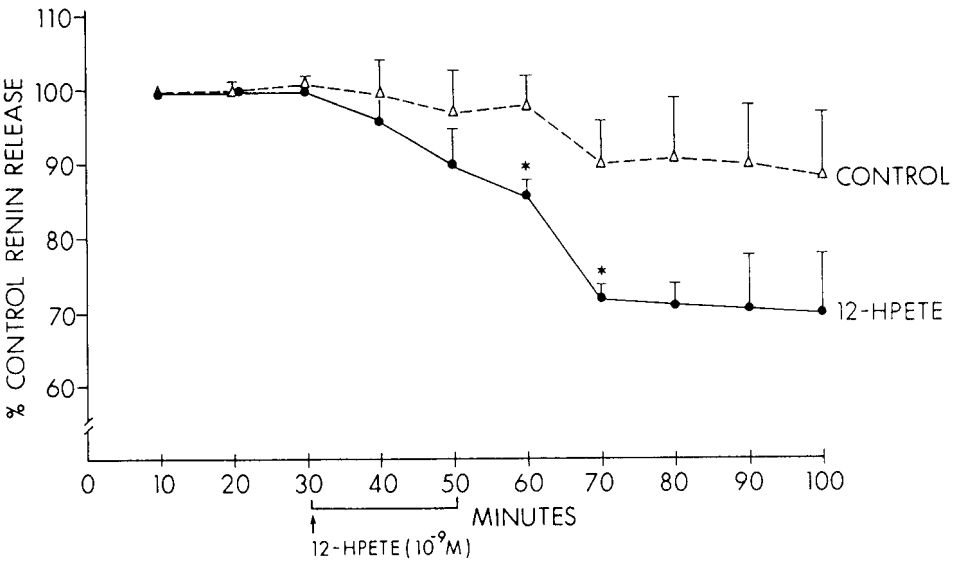

Figure 4. Effects of 12-HPETE in the perifusion system. Results are mean ± SE of five to six sets of separate experiments. 12-HPETE significantly inhibited renin release compared with control slices over the 60- to 70-min time period ($P < 0.05$).

group, failed to effect basal or iloprost-induced renin levels. Fourth, the HETES are not generally inhibitory in every cell. They act as mediators of glucose-induced insulin secretion in pancreatic islet cells (7) and angiotensin II-induced aldosterone secretion in adrenal glomerulosa cells (9). The actions of 12-HETE/HPETE on PGI_2 or iloprost-induced renin release are also not merely due to nonspecific hydroperoxide breakdown products, since mannitol, ascorbate, or diethyldithiocarbamate (14) did not alter basal or iloprost-induced renin secretion (our unpublished data).

The cellular mechanisms by which LO products

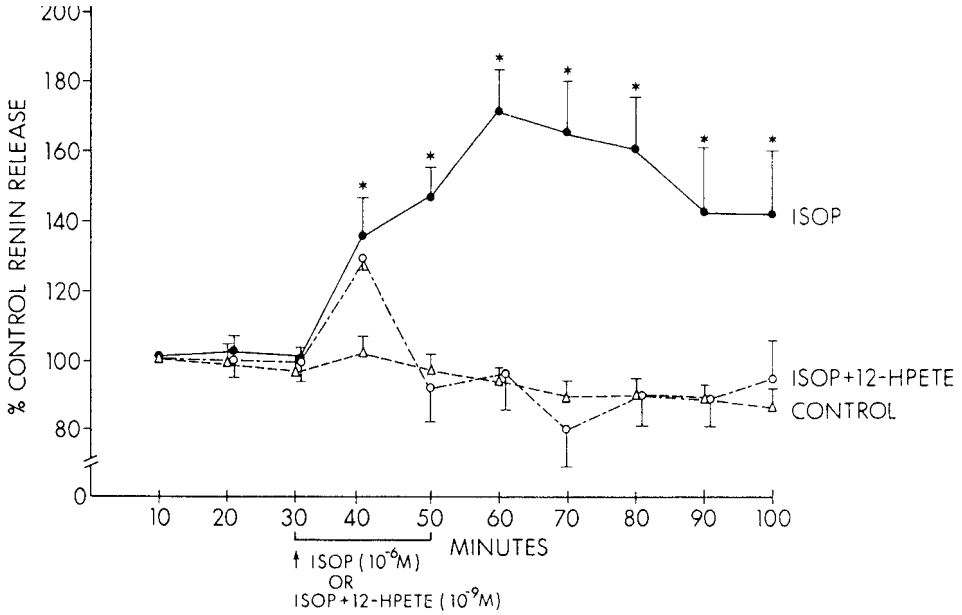

Figure 5. Effects of ISOP or ISOP + 12-HPETE in the perifusion system. Results are mean ± SE of four to five sets of separate experiments. ISOP significantly stimulated renin release compared with control slices over 40–100 min ($P < 0.05$). No significant differences were found between control and ISOP + 12-HPETE over 50–100 min.

inhibit PGI_2- or ISOP-mediated renin release are not clear. Renin release is regulated by both adenylate cyclase-cAMP as well as by the calcium-protein kinase C messenger systems. These systems probably act in a concerted fashion (1, 21). Although actions of PG on renin have been linked to adenylate cyclase systems (22), HETES can alter calcium flux, activate protein kinase C, and can be readily incorporated into membrane phospholipids, thereby altering membrane fluidity and the permeability of the cells to cations (23–25). Our studies indicate that both 12-HPETE and 12-HETE also block the effects of nifedipine (a calcium channel blocker)-induced renin secretion (unpublished). It is likely that generation and action of LO metabolites are interrelated with changes in calcium and stimulus secretion coupling in the juxtaglomerular cells.

The data presented here and our earlier studies (12, 26) suggest that dual pathways of AA metabolism are present in the kidney juxtaglomerular cells, which have opposing regulatory effects on renin release, a stimulatory CO cascade via PGI_2, and an inhibitory LO cascade. Moreover, 12-LO products from this cascade are more potent inhibitors of renin release on a molar basis than PGI_2 as a secretogogue. Similar results have been reported by Chansel et al. (27) using cultured human mesangial cells.

Our study demonstrates that HETES are potent modulators of PG actions on renin release. These results suggest that the concerted actions of these compounds could be critical determinants of juxtaglomerular cell (a modified vessel) renin secretion.

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