Renal arterial 20-hydroxyeicosatetraenoic acid levels: regulation by cyclooxygenase

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Cheng, Monica K., John C. McGiff, and Mairead A. Carroll. Renal arterial 20-hydroxyeicosatetraenoic acid levels: regulation by cyclooxygenase. Am J Physiol Renal Physiol 284: F474–F479, 2003. First published November 5, 2002; 10.1152/ajprenal.00239.2002.—20-HETE, a potent vasoconstrictor, is generated by cytochrome P-450 ω-hydroxylases and is the principal eicosanoid produced by preglomerular microvessels. It is released from preglomerular microvessels by ANG II and is subject to metabolism by cyclooxygenase (COX). Because low-salt (LS) intake stimulates the renin-angiotensin system and induces renal cortical COX-2 expression, we examined 20-HETE release from renal arteries (interlobar and arcuate and interlobular arteries) obtained from 6- to 7-wk-old male Sprague-Dawley rats fed either normal salt (0.4% NaCl) or LS (0.05% NaCl) diets for 10 days. With normal salt intake, the levels of 20-HETE recovered were similar in arcuate and interlobular arteries and interlobar arteries: 30.1 ± 8.5 vs. 24.6 ± 5.3 ng·mg protein⁻¹·30 min⁻¹, respectively. An LS diet increased 20-HETE levels in the incubate of either arcuate and interlobar or interlobar renal arteries only when COX was inhibited. Addition of indomethacin (10 μM) to the incubate of arteries obtained from rats fed an LS diet resulted in a two- to threefold increase in 20-HETE release from arcuate and interlobular arteries, from 30.1 ± 13.2 to 101.8 ± 42.6 ng·mg protein⁻¹·30 min⁻¹ (P < 0.03), and interlobar arteries, from 31.7 ± 15.1 to 61.9 ± 29.4 ng·mg protein⁻¹·30 min⁻¹ (P < 0.05) compared with release of 20-HETE when COX was not inhibited. An LS diet enhanced vascular expression of cytochrome P-4504A and COX-2 in arcuate and interlobular arteries; COX-1 was unaffected. Metabolism of 20-HETE by COX is proposed to represent an important regulatory mechanism in setting preglomerular microvascular tone.

renal microvessels; salt depletion; eicosanoids; cytochrome P-450

PREGLOMERULAR SMALL ARTERIES and arterioles (PGA) occupy a key position in the regulation of renal circulation (19) and are endowed with high levels of cytochrome P-450 (CYP) ωω-1 hydroxylase enzymes that generate 19- and 20-HETE (4). The latter is the putative mediator of renal autoregulation (30) and tubuloglomerular feedback (29) by virtue of its capacity to constrict PGA (15). The segmental distribution of 20-HETE synthetic ability in the renal vasculature demonstrates increases with decreasing vascular diameter (17). Thus the most important vascular segment for regulating renal vascular resistance and glomerular function, the afferent arteriole, is most heavily invested with 20-HETE synthetic ability (15).

20-HETE has been shown to be released from PGA by ANG II via activation of AT2 receptors (9). Several mechanisms govern the tissue levels and biological activity of 20-HETE, including glucuronide conjugation (22), incorporation into tissue phospholipids (3), and metabolism by cyclooxygenase (COX) to PG analogs that possess different biological properties from 20-HETE (6). For example, in the rabbit kidney, 20-HETE produces vasodilatation, an effect abrogated by inhibition of COX, suggesting transformation of 20-HETE to PG analogs possessing vasodilator properties (6).

Stimulation of the renin-angiotensin system (RAS) has been linked to induction of renal cortical COX-2 (28); viz, Na⁺ deprivation increased ANG II generation and induced cortical COX-2 expression. Because ANG II stimulates release of 20-HETE from PGA (9), we explored potential interactions involving 20-HETE and COX-2, under conditions of restricted intake of salt, in microdissected interlobar and arcuate and interlobular arteries of adult rats. We have reported that 20-HETE levels in incubates of renal microvessels, primarily afferent arterioles, of adult rats were increased after inhibition of COX (7).

The present study was conducted on microdissected arteries of 6- to 7-wk-old rats, because 20-HETE formation has been described to peak at this age (20). We separated interlobar arteries from arcuate and interlobular arteries to evaluate segmental variation in production of CYP-derived arachidonic acid (AA) metabolites. Because our preliminary study indicated the importance of COX-dependent transformation of 20-HETE (7), we determined segmental changes in COX-2 and COX-1 expression as well as that of ωω-1 hydroxylase CYP4A enzymes in interlobar arteries via a vis arcuate and interlobular arteries. We found that a low-salt (LS) diet increased release of 20-HETE from both sets of arteries, a response that was greater in arcuate and interlobu-
lar than interlobar arteries and was associated with increased COX-2 expression in the former. Inhibition of COX was required to demonstrate increased release of 20-HETE from PGA. Metabolism of 20-HETE by COX is proposed to represent an important regulatory mechanism in the preglomerular microcirculation that governs arterial-arteriolar tone.

MATERIALS AND METHODS

Preparation of Rat Kidney Microvessels

Male Sprague-Dawley rats (between 150 and 175 g) were divided into two groups, each receiving either normal salt (NS; 0.4% NaCl; n = 28) or LS (0.05% NaCl; n = 28) for 10 days. After this treatment, rats were anesthetized with pentobarbital sodium (60 mg/kg). Saline-perfused (20 ml) kidneys were isolated and freed from surrounding tissue. Each kidney was hemisected and placed in ice-cold PBS (Sigma). Interlobar arteries (200–250 μm) and arcuate (100–150 μm) and interlobar (60–80 μm) arteries were isolated by microdissection (Fig. 1). Arteries (interlobar arteries and arcuate and interlobar arteries) were washed three times with Tyrode solution (pH 7.4; Sigma) and treated as follows.

Release of 20-HETE and epoxyeicosatrienoic acids from PGAs

Renal vessels (interlobar arteries and arcuate and interlobar arteries) obtained from eight pairs of NS and LS groups were further divided into two groups, with one of them receiving either a normal salt (N) diet (0.4% NaCl; n = 8) or low-salt (LS) diet (0.05% NaCl; n = 8) for 10 days in the absence or presence of indomethacin (Indo; 10 μM). Arteries were incubated with 1 mM NADPH and 7 μM [14C]arachidonic acid for 30 min at 37°C. Samples were extracted and the supernates were separated by reverse-phase HPLC. Values are means ± SE. *P < 0.05.
Western Blot Analysis of COX-1 and -2 and CYP4A Proteins

Tissues were lysed with 10 mM Tris·HCl (pH 7.5) and 1% SDS, followed by centrifugation at 14,000 rpm for 15 min. Protein concentrations of supernates were determined by using a detergent-compatible Bio-Rad protein assay kit. Forty micrograms of cell lysate were mixed with an equal volume of 2× SDS-PAGE sample buffer, separated on a 10% SDS-PAGE gel, and transferred to nitrocellulose membranes. After blocking with 5% milk, membranes were probed with antibodies specific for COX(s) and CYP4A (polyclonal anti-goat COX-1 and COX-2 antibodies (Santa Cruz Biotechnology) and polyclonal anti-rabbit CYP4A antibody (Gentest)) for 1 h at room temperature. The membranes were washed with Tris-buffered saline with Tween 20 and incubated with the appropriate horseradish peroxidase-conjugated antisera. Proteins were detected by enhanced chemiluminescence and exposed to film for visualization.

Statistical Analysis

Results are expressed as means ± SE. Either a Student’s two-sample t-test or a nonparametric two-sample rank sum test was used to analyze differences between groups, depending on whether assumptions of normality were met. Paired analyses (paired t-test or Wilcoxon signed-rank test) were used when comparisons were made of data obtained from the same experimental preparation (i.e., arcuate and interlobular arteries vs. interlobar arteries from the same kidney). Unpaired analyses (unpaired t-test or Mann-Whitney U-test) were used when comparisons were made of data obtained from different experimental preparations (i.e., kidneys of NS vs. LS groups). A P value of <0.05 was considered significant.

RESULTS

Release of HETEs from Renal Microvessels

We had reported that rat glomerular arterioles/arteries (afferent, arcuate and interlobular, and interlobar), obtained by using the iron oxide method, generate relatively large quantities of 20-HETE and lesser quantities of 19-HETE (9). Metabolism of AA in arcuate and interlobular arteries (60- to 150-μm inner diameter) was compared with that in interlobar arteries (200- to 250-μm inner diameter) as affected by LS (0.05%; n = 8) vs. NS (0.4%; n = 8) intake for 10 days in the presence or absence of indo (10 μM) to inhibit COX. On the basis of GC-MS single ion monitoring analyses, 20-HETE was the principal product of all renal arteries/arterioles exceeding 19-HETE release by ninefold; 16-, 17-, and 18-HETEs, which have been reported to be released from the kidney by ANG II (4), were not detected.

NS diet. Under conditions of NS intake, and in the absence of Indo, metabolism of [14C]AA (7 μM) to 20-HETE, analyzed with HPLC, was similar in arcuate and interlobular arteries and interlobar arteries as reflected in their capacity to release 20-HETE: 30.1 ± 8.5 vs. 24.6 ± 5.3 ng·mg protein⁻¹·30 min⁻¹, respectively (Fig. 2A). After inhibition of COX, recovery of 20-HETE from the incubate of arcuate and interlobular arteries increased by approximately twofold, to 62.2 ± 25.0 ng·mg protein⁻¹·30 min⁻¹ (P < 0.05), whereas release of 20-HETE from interlobar arteries was not affected (Fig. 2A).

LS diet. Unless COX was inhibited, an LS diet did not increase 20-HETE levels from either arcuate and interlobular or interlobar renal arteries compared with recovery from these arteries obtained from rats on an NS diet (Fig. 2A). In rats on an LS diet, addition of Indo to the incubate containing either arcuate and interlobular or interlobar arteries resulted in a two- to threefold increase in 20-HETE recovery, 39.1 ± 13.2 to 101.8 ± 42.6 ng·mg protein⁻¹·30 min⁻¹ (P < 0.03) from arcuate and interlobular arteries and, for interlobar arteries, from 31.7 ± 15.1 to 61.9 ± 29.4 ng·mg protein⁻¹·30 min⁻¹ (P < 0.05) compared with a failure to increase 20-HETE recovery from the incubate containing PGA when COX was not inhibited (Fig. 2A).

That is, when COX was not inhibited, recovery of 20-HETE from the incubate of either arcuate and interlobar or interlobar arteries microdissected from rats on an LS diet was reduced by ~50–60% (Fig. 2A). On the basis of GC-MS analyses, the profile of CYP-HETEs formation was not altered by salt depletion; i.e., 20-HETE was the principal HETE formed, 19-HETE being one-ninth or less abundant (data not shown).

Release of Epoxyeicosatrienoic Acids from Renal Microvessels

Basal epoxyeicosatrienoic acid (EET) release from arcuate and interlobular and interlobar arteries did not differ (Fig. 2B) and was comparable to basal release of 20-HETE from these arteries, ~20–30 ng·mg protein⁻¹·30 min⁻¹. Moreover, vascular EET recovery was not affected by either COX inhibition or decreased salt intake (Fig. 2B).

Cytochrome CYP4A Hydroxylase Expression

To determine whether changes in renal CYP4A hydroxylase expression occurred concomitantly with changes in release of 20-HETE from renal arteries, immunoblot analyses were conducted on arcuate and interlobular and interlobar arteries obtained from rats subject to either NS or LS intake for 10 days. In rats on NS intake, Western immunoblotting disclosed that CYP4A expression in arcuate and interlobular arteries was greater than that in interlobar arteries (Fig. 3); whereas on LS intake, CYP4A protein was increased only in interlobar arteries.

COX Expression

As 20-HETE recovery from the incubate was increased by COX inhibition with Indo and as renal cortical COX-2 expression is increased by salt restriction, we examined whether COX-2 expression was increased in PGA in response to an LS diet. In rats fed an NS diet, COX-2 was expressed at low levels in both sets of arteries (Fig. 4). When dietary salt was restricted, COX-2 expression was significantly increased in arcuate and interlobar arteries (Fig. 4), whereas expression of COX-1 was unaffected by salt depletion (Fig. 5).
DISCUSSION

We addressed possible links among stimulation of RAS, 20-HETE synthesis, and induction of COX-2. As an LS diet activates RAS and as ANG II increases 20-HETE release from PGA (9), we had postulated that 20-HETE released from PGA in response to an LS diet would be increased. We quantitated CYP-AA metabolism and determined CYP4A and COX(s) expression and activity in microdissected preglomerular arterial elements (interlobar and arcuate and interlobular arteries) obtained from NS- and LS-treated rats. With NS intake, renal COX-2 is low in the rat kidney and was not detected either in PGA or glomeruli (28), whereas an LS diet enhanced 20-HETE recovery from PGA by two- to threefold and induced expression of CYP4A and COX-2. However, increased release of 20-HETE from PGA in response to an LS diet required COX inhibition to be demonstrated, indicating that COX serves as a metabolic pathway for 20-HETE by forming PG analogs of 20-HETE. These findings are in accordance with our proposal that metabolism of 20-HETE by COX-2 acts as a braking mechanism that prevents the unopposed action of 20-HETE, a potent constrictor of renal microvessels (15). In contrast to increased formation of 20-HETE in response to an LS diet, EET production was unaffected. Increased intake of salt is reported to selectively elevate epoxygenase activity (2). The present study extends the interactions of pressor hormones with eicosanoids and supports the concept that the coordinate interaction of \( \Delta^6 \)-hydroxy-lase and COX-2 participates in the regulation of glomerular hemodynamics in LS states.

20-HETE is the principal eicosanoid in PGA (9). In the initial studies of the renal CYP pathway, 20-HETE was reported to be the most abundant product of renal AA metabolism in microsomes prepared from the whole kidney (24). 20-HETE was subsequently identified as the dominant arachidonate metabolite in crucial sites intrarenally: afferent arterioles and contiguous microvessels, proximal tubules, and thick ascending limb (8, 21). Prominent among the activities of 20-HETE is its capacity to selectively elevate epoxyenase activity. The present study extends the interactions of pressor hormones with eicosanoids and supports the concept that the coordinate interaction of \( \Delta^6 \)-hydroxy-lase and COX-2 participates in the regulation of glomerular hemodynamics in LS states.
action when it is given to the intact kidney (26). The most compelling evidence for a pathological role for 20-HETE is in the renal failure produced by hepatic cirrhosis (hepatorenal syndrome), which is characterized by intense renal vasoconstriction (11). Sacerdoti et al. (23) have shown that 20-HETE is increased greatly in the hepatorenal syndrome, exceeding by threefold the excretion of thromboxane, the predominant renal COX product in patients with cirrhosis and ascites.

Of the mechanisms that lower the potency of the vasoconstrictor action of 20-HETE, thereby blunting the renal circulatory response to the eicosanoid, metabolism by COX to PG analogs (25) having either lesser vasoconstrictor activity or even vasodilator activity may play an important role under conditions of salt deprivation. The facile metabolism of 20-HETE by COX was first recognized by Escalante et al. (12), who prevented the contractile response of aortic rings to 20-HETE by either inhibition of COX or blockade of the endoperoxide/thromboxane receptor, indicating that under these experimental conditions 20-HETE is transformed by COX to an analog of either PGH₂ or thromboxane A₂.

ANG II, in addition to promoting 20-HETE production by PGA, induces expression of COX-2 in the medullary thick ascending limb (13). In this instance, ANG II acts through stimulation of TNF-α to increase COX-2 activity (14). In defining the 20-HETE-dependent mechanism responsible for the renal microcirculatory response to salt deprivation, an essential component has been demonstrated, viz, conversion of 20-HETE by COX-2 to products, PG analogs of 20-HETE (5). The coexpression of an inducible membrane-associated PGE₂ synthase (18) that acts in concert with COX-2 may favor formation of 20-OH PGE₂, a vasodilator PG analog of 20-HETE (5). The capacity of COX to metabolize 20-HETE to PG analogs, for example, 20-OH PGF₂α and 20-OH PGE₂, (25) may be critical to the modification of the renal vascular and tubular actions of ANG II in states of salt deprivation or abnormalities of salt and water homeostasis, such as hepatic cirrhosis, heart failure, and diabetic and hypertensive nephropathy (10). In these conditions, a large component of renal blood flow is COX-2 dependent, as evidenced by the ability of NSAI ds to depress renal blood flow only when pathophysiological conditions prevail, producing COX-2 expression. These factors are clearly seen when comparing the dog at rest to one challenged by surgical stress (27). In the resting dog, Indo, even in toxic doses, does not affect renal blood flow, whereas in the dog subject to trauma, which elevates renal PG production and plasma renin activity (via a COX-2-dependent mechanism), Indo acutely reduces elevated renal PG levels associated with a corresponding sharp decrease in renal blood flow.

In summary, 20-HETE recovery from PGA was shown to vary segmentally. When rats were challenged with an LS diet, arcuate and interlobular arteries exhibited the greatest release of 20-HETE, exceeding those of interlobar arteries under conditions of both NS and LS intake. These differences required inhibition of COX-2 to be observed, because the omission of Indo resulted in 20-HETE levels that did not differ between arcuate and interlobular and interlobar arteries, irrespective of salt intake (Fig. 2). In contrast, EET release from both small or large arteries was unaffected by either NS or LS intake, despite inhibition of COX.

On the basis of the present study, we hypothesize that major adverse effects of aspirin-like drugs may result from the direct vasoconstrictor action of 20-HETE. That is, when 20-HETE cannot be transformed to PG analogs under conditions of salt depletion, the unopposed action of 20-HETE constricts the renal vasculature. This hypothesis has received support from the recent demonstration that two of the principal 20-HETE PG analogs, 20-OH PGE₂ and 20-OH PGF₂α, dilate the rat renal vasculature (5). The present study provides a scaffolding for examining a potentially key regulatory system operating within PGA, particularly when extracellular fluid volume is contracted, initiating a compensatory mechanism involving COX-2 that mitigates the constrictor effect of 20-HETE on renal microvessels.

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