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Deficiency of COX-1 causes natriuresis and enhanced sensitivity to ACE inhibition

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Deficiency of COX-1 causes natriuresis and enhanced sensitivity to ACE inhibition.

Background. Prostanoid products of the cyclo-oxygenase (COX) pathway of arachidonic acid metabolism modulate blood pressure (BP) and sodium homeostasis. Conventional non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit both COX isoforms (COX-1 and -2), cause sodium retention, exacerbate hypertension, and interfere with the efficacy of certain anti-hypertensive agents such as angiotensin-converting enzyme (ACE) inhibitors. While a new class of NSAIDs that specifically inhibit COX-2 is now widely used, the relative contribution of the individual COX isoforms to these untoward effects is not clear.

Methods. To address this question, we studied mice with targeted disruption of the COX-1 (*Ptgs1*) gene. Blood pressure, renin mRNA expression, and aldosterone were measured while dietary sodium was varied. To study interactions with the renin-angiotensin system, ACE inhibitors were administered and mice with combined deficiency of COX-1 and the angiotensin II subtype 1A (AT_{1A}) receptor were generated.

Results. On a regular diet, BP in COX-1−/− mice was near normal. However, during low salt feeding, BP values were reduced in COX-1−/− compared to +/+ animals, and this reduction in BP was associated with abnormal natriuresis despite appropriate stimulation of renin and aldosterone. Compared to COX-1+/+ mice, the actions of ACE inhibition were markedly accentuated in COX-1−/− mice. Sodium sensitivity and BP lowering also were enhanced in mice with combined deficiency of COX-1 and AT_{1A} receptor.

Conclusions. The absence of COX-1 is associated with sodium loss and enhanced sensitivity to ACE inhibition, suggesting that COX-1 inhibition does not cause hypertension and abnormal sodium handling associated with NSAID use.

Key words: prostaglandins, mouse, gene targeting, renin, angiotensin II, NSAIDs, antihypertension, analgesics.

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The cyclo-oxygenase (COX) pathway for arachidonic acid metabolism produces prostaglandins and thromboxanes [1]. These lipid mediators have a range of biological actions that are relevant to blood pressure homeostasis including regulation of vascular tone [2] and modulation of sodium excretion [3, 4]. Two COX isoforms share similar enzymatic properties but differ markedly in their pattern and regulation of expression [5]: COX-1 is constitutively expressed in most tissues whereas the expression of COX-2 is regulated and can be induced to high levels by cytokines and growth factors [6]. While actions of cyclo-oxygenase metabolites to influence blood pressure have been documented [3], the individual roles of the COX isoenzymes in physiological regulation of blood pressure have not been clearly defined.

Some of the evidence supporting a role for the COX pathway in regulating blood pressure and kidney function has come from clinical experiences with non-steroidal anti-inflammatory drugs (NSAIDs). The pharmacological mechanism of action of NSAIDs is based on their inhibition of COX isoforms [5] and conventional NSAIDs inhibit both COX-1 and COX-2 [5, 7]. In susceptible patients, COX inhibition by NSAIDs may cause acute renal failure, edema, and hypertension [8, 9]. In addition, NSAIDs may cause resistance to certain antihypertensive therapies, especially angiotensin-converting enzyme (ACE) inhibitors [10, 11]. However, the relative contributions of COX-1 and COX-2 inhibition to these adverse effects of NSAIDs are not clear. Recently, NSAIDs with specificity for COX-2 have been introduced for clinical use. These agents appear to have analgesic actions that are equivalent to conventional NSAIDs, but they cause fewer gastrointestinal toxicities. However, there is some lack of consensus in clinical studies regarding the effects of COX-2 inhibitors on blood pressure regulation and responses to antihypertensive therapy. Moreover, the role of COX-1 in blood pressure homeostasis is unknown.

To study the role of COX-1 in the regulation of blood

pressure, a mouse cell line with targeted disruption of the COX-1 (*Ptgs1*) gene was used [12]. Our current study documents actions of COX-1 to maintain blood pressure and sodium balance and suggests that inhibition of COX-1 would have favorable effects in hypertension.

METHODS

Mice

The generation of COX-1 and angiotensin II subtype 1A (AT_{1A}) receptor-deficient mice has been described previously [12, 13]. The COX-1-deficient mice were bred to the AT_{1A} receptor-deficient strain to generate double mutant COX-1 and AT_{1A} animals. Mice were screened for the appropriate targeted gene mutations by Southern blot or polymerase chain reaction (PCR) analysis of tail genomic DNA as previously described [12, 13]. Animals were bred and maintained in the animal facility of the Durham VAMC under National Institutes of Health (Bethesda, MD, USA) guidelines.

Systolic blood pressure measurements in conscious mice

Systolic blood pressures were measured in conscious mice using a computerized tail cuff system (Visitech Systems, Cary, NC, USA) that determines systolic blood pressure using a photoelectric sensor [14]. This system allows pressures to be measured in four mice simultaneously and minimizes the potential for observer bias. Before the study was initiated, mice were adapted to the apparatus for at least five days. The validity of this system has been established previously [14] and we have demonstrated its correlation with intra-arterial pressure measurements in several experimental systems [13, 14].

Renin mRNA expression in mouse kidneys

To examine the effects of the COX-1 mutation on the activity of the renin-angiotensin system (RAS), renin mRNA expression was analyzed in kidney cortex from wild-type ($N = 17$) and COX-1$-/-$ mice ($N = 16$) using a ribonuclease protection assay as described previously [15]. Kidneys were harvested, rapidly frozen in liquid nitrogen, and total RNA was isolated using the guanidinium/isothiocyanate method. Riboprobes labeled with ^{32}P were transcribed from a 290 bp fragment from exon 9 of the mouse renin gene [16]. The riboprobe was hybridized in solution with total RNA from the kidney specimens. Following RNase treatment, the protected fragments were precipitated with trichloroacetic acid (TCA), filtered through a glass filter, and ^{32}P cpm was determined with a scintillation counter. To estimate the concentration of renin mRNA in each sample, a standard curve was constructed by hybridizing the ^{32}P-labeled renin probe with known quantities of renin cDNA that had been transcribed in vitro. Renin mRNA levels were expressed as pg of renin mRNA/μg total kidney RNA.

Effects of reduced dietary sodium on systolic blood pressures

To determine the effects of the COX-1 gene disruption on the adaptation to a reduced dietary sodium intake, systolic blood pressures were measured in mice that were sequentially fed diets differing in sodium chloride content. COX-1$+/+$ ($N = 8$) and COX-1$-/-$ ($N = 12$) mice were first fed a control diet containing 0.4% sodium chloride for 14 days. This was followed by a 14-day period in which the animals were fed a low salt diet containing $<0.02\%$ sodium chloride. Diets were purchased from Harlan-Teklad (Madison, WI, USA). Mice were allowed free access to water. Systolic blood pressures were measured at least five times per week throughout the period of study.

Measurements of urinary sodium and aldosterone excretion

To estimate urinary excretion of sodium and aldosterone, mice were individually housed in specially designed metabolic cages that accommodate individual mice [17]. Separate groups of COX-1$+/+$ ($N = 7$) and $-/-$ ($N = 6$) mice were fed a normal salt (0.4% NaCl) diet followed by a low salt ($<0.02\%$ NaCl) diet. Twenty-four-hour urine specimens were collected and urine sodium was measured with a flame photometer (Instrumentation Laboratory, Lexington, MA, USA). The 24-hour urine sample on day 7 of each diet period was collected and aldosterone concentration was measured by radioimmunoassay according to the manufacturer's instructions (Diagnostic Labs, Los Angeles, CA, USA).

Effects of ACE inhibition in COX-1-deficient mice

To examine the effect of reducing angiotensin II levels on blood pressure, COX-1$+/+$ ($N = 10$) and $-/-$ mice ($N = 8$) were treated with enalapril 30 mg/kg/day by gavage for 14 days. Systolic blood pressures were measured during treatment.

Statistical analysis

The values for each parameter within a group are expressed as the mean ± standard error of the mean (SEM). For comparisons between groups, statistical significance was assessed using an unpaired *t* test. A paired *t* test was used for comparisons within groups. Survival analysis during low salt feeding was determined by chi-square.

RESULTS

Cyclo-oxygenase-1–deficient mice have normal blood pressures and stimulated renin expression

To determine the effects of COX-1 deficiency on basal blood pressure regulation, systolic blood pressures in wild-

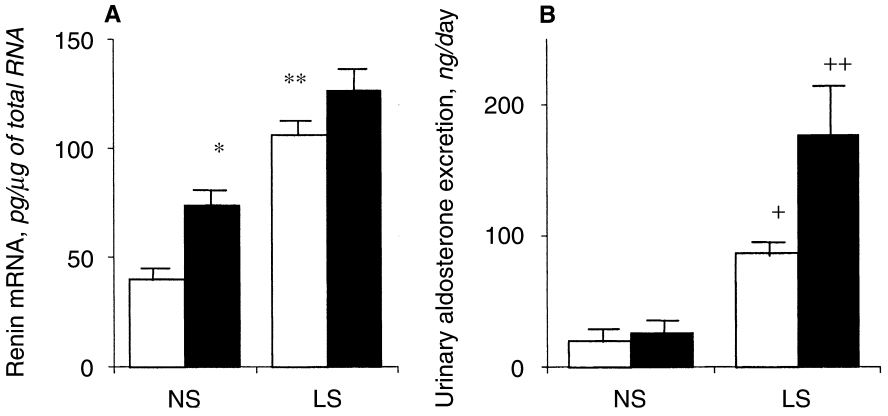

Fig. 1. (*A*) Renin mRNA expression in renal cortex. Renin mRNA expression levels were significantly higher in COX-1 deficient mice compared to wild type controls. During low salt feeding, renin expression tended to be higher in COX-1 deficient mice, though this difference did not achieve statistical significance ($P = 0.37$). Data are presented as the mean ± SEM (pg/μg of total RNA). Dietary periods: 0.4% NaCl normal salt (NS); <0.02% NaCl low salt (LS). *$P = 0.01$ vs. wild type controls; **$P < 0.01$ vs. NS. (*B*) Effect of dietary sodium intake on urinary aldosterone excretion measured in 24-hour urine collection from wild-type mice and COX-1 deficient mice. Urinary aldosterone excretion tended to be higher in COX-1 deficient mice than wild-type mice on normal diet and low salt diet but this difference did not achieve statistical significance. Urinary aldosterone excretion increased significantly during low salt feeding in both groups. Data are presented as the mean ± SEM (ng/day). +$P < 0.001$ vs NS; ++$P = 0.03$ vs. NS. Symbols are: (□) wild-type controls; (■) COX-1 deficient mice.

type and COX-1−/− mice on a conventional (0.4% NaCl) diet were compared. On this dietary regimen, systolic blood pressures tended to be lower in COX-1-deficient mice (96 ± 2 mm Hg) than control animals (102 ± 3 mm Hg; $P = 0.13$), but this difference was not statistically significant. Under similar conditions, renin mRNA expression in renal cortex was also compared in COX-1 +/+ and −/− animals. As shown in Figure 1, renin mRNA expression was significantly higher in the COX-1-deficient animals (74 ± 9 pg/μg total RNA) than controls (40 ± 6 pg/μg total RNA; $P = 0.01$). Urinary aldosterone excretion also tended to be higher in COX-1−/− (26 ± 5 ng/day) than in wild-type animals (20 ± 4 ng/day), but this difference did not achieve statistical significance ($P = 0.35$).

Cyclo-oxygenase-1 contributes to maintenance of blood pressure during low salt feeding

Based on our findings that the absence of COX-1 is associated with stimulation of the RAS, we posited that COX-1 might have a role in regulating sodium homeostasis. To examine this possibility, we compared blood pressure and RAS activity in wild-type and COX-1-deficient animals during dietary sodium depletion. As shown in Figure 3, after 14 days on a diet that is very low in sodium (<0.02% NaCl), blood pressures were significantly lower in COX-1-deficient mice (93 ± 2 mm Hg) than in wild-type controls (101 ± 3 mm Hg; $P = 0.04$). Figure 1 shows that during low salt feeding, renin mRNA expression in the renal cortex increased substantially in both groups and tended to be higher in the COX-1−/− mice (125 ± 10 pg/μg total RNA) than controls (106 ± 13 pg/μg total RNA), but this difference did not achieve statistical significance ($P = 0.37$); urinary aldosterone excretion followed a similar pattern (Fig. 1). Aldosterone excretion increased significantly in both groups during low salt feeding ($P = 0.001$ vs. baseline for COX-1+/+ and $P = 0.03$ versus baseline for COX-1−/−) and tended to be higher in the COX-1−/− animals (176 ± 51 ng/day) compared to wild-type controls (86 ± 16 ng/day; $P = 0.14$). Upon institution of the low salt diet, urine sodium excretion fell dramatically in both groups. However, urine sodium excretion was higher in COX-1−/− animals than controls. For example, on day 3 during the adaptation period to the new diet, urine sodium excretion was threefold higher in the COX-1-deficient mice (30 ± 12 mEq/day) than controls (10 ± 4 mEq/day; $P = 0.05$), despite similar food intake.

Blood pressure response to ACE inhibition is exaggerated in COX-1-derficient mice

In view of the enhanced renin expression in COX-1-deficient mice and reports suggesting that conventional NSAIDs interfere with the actions of ACE inhibitors, blood pressure responses to ACE inhibition were compared in COX-1+/+ and −/− animals fed the control (0.4% NaCl) diet. Mice were treated with enalapril 30 mg/kg/day by gavage for 14 days and systolic blood pressures were measured. The ACE inhibitor reduced blood pressures in both groups. However, the hypotensive effects of ACE inhibition were much more marked in the COX-1-deficient animals than controls. Following enalapril treatment, systolic blood pressures were markedly lower in COX-1−/− mice (71 ± 3 mm Hg) than controls (85 ± 3 mm Hg; $P < 0.01$; Fig. 2). These data suggest that the stimulated renin expression in the COX-1 deficient animals is a compensatory response to maintain blood pressure near normal levels.

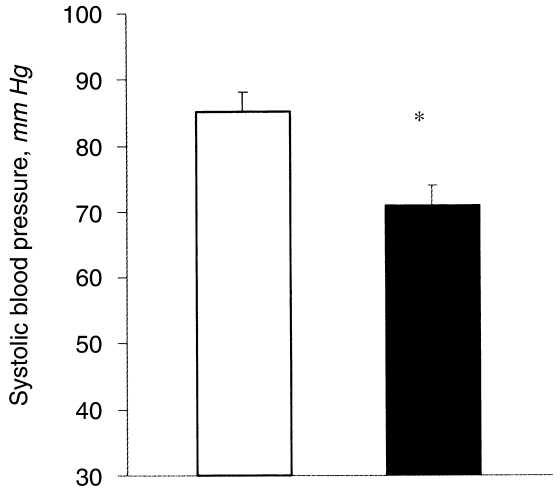

Fig. 2. Blood pressure response to angiotensin-converting enzyme (ACE) inhibition. Systolic blood pressures were markedly lower in COX-1 deficient mice treated with enalapril (■) than wild-type controls (□). Data are presented as mean ± SEM (mm Hg). *P = 0.01 vs wild type controls.

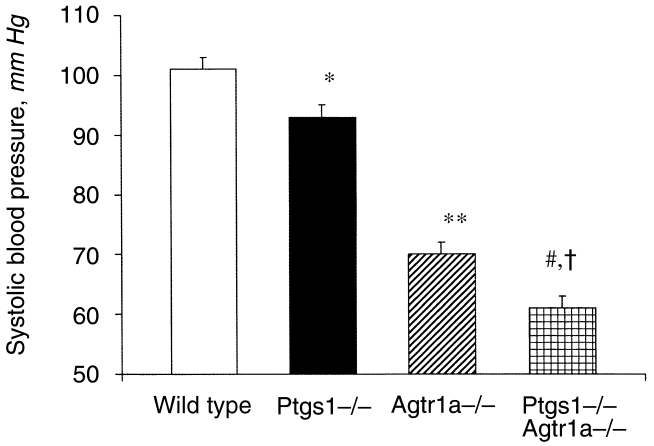

Fig. 3. Influence of low salt feeding on blood pressure. Blood pressures were lower in COX-1 deficient mice (Ptgs1−/−) compared to wild-type controls during low salt feeding. AT_{1A} receptor-deficient mice (Agtr1a−/−) also had a decrease in blood pressures while on the low salt diet. Blood pressures values were further reduced in mice with combined deficiency in both COX-1 and AT_{1A} receptor (Ptgs1−/− Agtr1a−/−). In addition, 25% of Ptgs1−/−Agtr1a−/− mice died during low salt feeding. Data are presented as mean ± SEM (mm Hg). *P = 0.04 vs. wild type controls; **$P < 0.001$ vs. wild-type controls, #P = 0.02 vs. Agtr1a−/−; †P = 0.03 survival during low salt feeding vs. Ptgs1−/−.

Mice with combined deficiency of COX-1 and AT_{1A} angiotensin receptors are markedly sensitive to sodium depletion

To further examine the interactions of the COX-1 pathway with the RAS and the mechanism of the exaggerated effect of ACE inhibition in COX-1 deficient mice, we produced mice with combined deficiency of COX-1 and the AT_{1A} angiotensin receptor, the major murine AT_1 receptor isoform. As we have previously reported [13], blood pressures are significantly reduced in mice lacking AT_{1A} receptors (91 ± 3 mm Hg; P = 0.02 vs. wild-type). Blood pressures were significantly lower in the COX-1 and AT_{1A} double knockout animals (84 ± 3 mm Hg; $P < 0.001$ versus COX-1−/− alone). Following low salt feeding, blood pressures fell in the AT_{1A} receptor-deficient animals (70 ± 3 mm Hg; $P <$ 0.001 vs. control diet). The double knockout animals experienced an even more marked fall in blood pressure on the low salt diet (61 ± 3 mm Hg; $P < 0.001$ vs. COX-1−/−, P = 0.02 vs. AT_{1A} receptor-deficient mice; Fig. 3). Moreover, during the period of low salt feeding, 25% of the double knockout animals developed marked reductions in blood pressure, became lethargic and died. There were no deaths in any of the other experimental groups during low salt feeding (P = 0.03 by chi-square vs. COX-1−/−).

DISCUSSION

Metabolites produced from arachidonic acid by the COX pathway mediate diverse physiological processes including pain, fever, inflammation, and thrombosis [1]. In addition, biological actions of COX metabolites are germane to blood pressure and sodium homeostasis [2, 18]. For example, prostaglandin (PG) E_2 and prostacyclin (PGI_2) are vasodilators, while thromboxane (TX) A_2 is a potent vasoconstrictor [18]. Although effects of prostanoids upon vascular tone may acutely alter blood pressure or regional perfusion, regulation of sodium excretion by the kidney is the critical determinant of long-term blood pressure homeostasis [19]. In this regard, COX metabolites also influence kidney functions by modulating glomerular filtration rate, renal blood flow, and excretion of salt and water [4].

Both COX isoforms are expressed in the kidney. Prominent expression of COX-1 has been reported in glomerular mesangial cells [20] and cortical collecting segments [20, 21] while COX-2 is expressed in cortical thick ascending limb of the loop of Henle [20, 22] and in the macula densa [20, 23]. As in other tissues, COX-1 expression in the kidney is largely constitutive [6, 20, 21]. On the other hand, COX-2 expression is markedly up-regulated by dietary sodium depletion [20, 23, 24]. In the macula densa, COX2 regulates renin release in chronic sodium depletion [23, 25, 26]. COX-2 also may be involved in the short loop feedback response whereby angiotensin II regulates renin release [26]. By contrast, in our studies there was no indication of a significant role for COX-1 to regulate renin expression during sodium depletion.

In a conventional environment, COX-1-deficient mice have a relatively mild phenotype that includes impaired platelet aggregation and reduced fertility [12]. Unlike COX-2-deficient mice [27, 28], kidney structure and mor-

phology is normal in COX-1−/− animals [12]. When they are maintained on standard 0.4% NaCl chow, we find that blood pressures of COX-1−/− mice are slightly lower although not significantly different from controls. However, despite their normal blood pressure, renin expression is significantly increased in kidneys of COX-1-deficient animals. This activation of the RAS seems to have functional significance, since the hypotensive response to ACE inhibition is significantly augmented in sodium-replete COX-1−/− mice compared to controls. Thus, the absence of COX-1 promotes a fall in blood pressure. In COX-1−/− mice, the RAS seems to be activated as a compensatory mechanism to help maintain normal blood pressure. Without this compensatory effect, the blood pressure lowering actions of COX-1-deficiency might be more prominent. Because of its expression in the macula densa and its role in regulation of renin release, we speculate that COX-2 may be involved in these compensatory actions. While a precise mechanism for these regulatory actions has not been identified, administration of COX-2 inhibitors to human is associated with a disproportionate reduction in prostacyclin synthesis [29, 30] and prostacyclin is a potent secretagogue for renin [31]. Finally, our findings suggest that the actions of conventional NSAIDs to interfere with the antihypertensive actions of ACE inhibitors are not due to inhibition of COX-1 and may, therefore, be related to COX-2 inhibition.

With sodium depletion, a significant difference in blood pressure is uncovered between the COX-1−/− and wild-type animals. Moreover, during dietary sodium restriction, the reduced blood pressure in COX-1-deficient mice was associated with exaggerated natriuresis. This abnormal natriuresis was not due to impaired activation of the RAS, since renin expression and aldosterone production were appropriately stimulated by the sodium-deficient diet and the absolute levels of renin mRNA and aldosterone excretion were actually highest in the COX-1−/− animals.

Our studies suggest that prostanoids produced by COX-1 have non-redundant actions to regulate blood pressure that are particularly important when dietary sodium intake is limited. Further, the absence of COX-1 cannot be compensated for by the presence of COX-2 in the COX-1−/− mice. Since the enzymatic functions of the two COX isoforms are virtually identical [5] and because of the importance of the kidney in chronic blood pressure regulation, the unique functions of COX-1 in blood pressure homeostasis may be related to its distinct pattern of expression in the kidney [20, 21]. Alternatively, discrete coupling between COX isoforms and synthesis of specific eicosanoid moieties has been suggested in previous studies [29, 30, 32]. Because prostanoids often function in an autocrine/paracrine fashion, expression and activity of COX-1 within specific nephron segments could produce circumscribed, local physiological actions.

Prostanoids mediate their biological effects by activating G protein-coupled receptors [18]. Several classes of prostanoid receptors are expressed in the kidney and these receptors have been linked to epithelial and hemodynamic functions. EP (E prostanoid) receptors for PGE_2 are expressed on epithelia in proximal and distal nephron segments [33–35]. In the kidney, stimulation of EP receptors modulates sodium and water flux [4]. Similarly, thromboxane (TP) receptors are expressed in the distal nephron [36] and these receptors stimulate tubuloglomerular feedback responses [37, 38]. The specific receptors that mediate the actions of COX-1 to alter blood pressure regulation cannot be determined from our studies. However, in previous studies, we have observed reduced blood pressures and altered sodium handling in mice deficient in various EP receptor isoforms. For example, we find that blood pressure is reduced in EP1- [39] and EP2-deficient mice [40]. Since PGE_2 is one of the major prostanoids produced by the kidney [41], it is possible that the absence of COX-1 is associated with reduced regional production of PGE_2 and consequent lack of stimulation of EP receptor isoforms, such as EP1 or EP2.

Interactions between the COX pathway and the renin-angiotensin system have been long recognized [2]. Our studies have identified a novel, cooperative interplay between these two systems to maintain blood pressure during dietary sodium depletion. We have previously reported that the absence of the major murine AT_1 angiotensin receptor (AT_{1A}) is associated with defective sodium handling [17]. Our analysis of mice with combined deficiency of COX-1 and AT_{1A} receptors indicates that during low salt feeding, in the absence of AT_{1A} receptors, COX-1 assumes a critical compensatory role. When this function is absent, adaptation to sodium depletion is impaired further, resulting in volume depletion and severe hypotension that can be fatal. The COX and RAS are both activated when effective arterial volume is reduced, as in fluid volume depletion, congestive heart failure, and cirrhosis [8, 9]. In these circumstances, it has been suggested that enhanced production of prostanoids attenuates the vasoconstrictor and anti-natriuretic effects of angiotensin II [2]. Our data indicate that COX-1 has an alternative function in these circumstances: to promote renal sodium retention and to support blood pressure.

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