Cyclooxygenase (COX) converts arachidonate to prostaglandin (PG) H$_2$ (PGH$_2$)$_1$ which can activate thromboxane-PGH$_2$ receptors. However, in vivo, PGH$_2$ is promptly metabolized to several biological active PGs and thromboxane A$_2$ (TxA$_2$). COX produces both vasoconstrictor and vasodilator metabolites. Therefore, its role in the regulation of BP and hemodynamics is difficult to predict and may explain the positive and negative studies regarding the effect of nonselective COX inhibitors (nonsteroidal anti-inflammatory drugs [NSAIDs]) on blood pressure (BP) in human. However, a metaanalysis showed a 5.0-mm Hg increase in the BP in NSAID users.$^5$

COX-2 is inducible,$^4$–$^6$ but in the kidney it is expressed constitutively in vascular endothelial cells, afferent arterioles, cortical thick ascending limbs, macula densa cells, and glomeruli.$^7$–$^8$ COX-2 regulates renin secretion.$^7$ COX-1 is expressed in the extraglomerular and intraglomerular mesangium, the terminal distal convoluted tubule, collecting duct, and vascular endothelial cells.$^9$–$^{10}$ Previous studies by Athirakul et al.$^1$–$^3$ have shown that COX-1 gene–deficient (COX-1$^{-/-}$) mice fail to conserve salt, leading to an exaggerated decline in the mean arterial pressure (MAP) during salt restriction. They also have diminished pressor and renal vasconstrictor responses during an acute infusion of angiotensin II.$^{12}$ These studies suggest that COX-1 may be required to maintain BP during states of high angiotensin II. However, less is known concerning the role of COX-1 during states of normal or low angiotensin II. This study was conducted in mice consuming a normal salt diet.

A loss of the normal reduction in BP during sleep (“nondipping” status) increases the risk for cardiovascular disease,$^{13}$–$^{16}$ but the mechanism is not clear. The decline in nocturnal BP is associated with a reduced sympathetic nervous system tone.$^{17}$ Loss of the nocturnal decline in BP is accompanied by reduced physical activity,$^{18}$ increased...
NaCl reabsorption,19,20 and impaired endothelial function.13 Because COX-1 may regulate NaCl reabsorption and endothelial function, we assessed the sleep-to-awake BP pattern in these mice using telemetry to test the hypothesis that COX-1 products mediate the normal circadian fluctuation in BP.

The first aim of this study was to determine the role of COX-1 in the regulation of BP and renal hemodynamics. The MAP was measured by telemetry in unanesthetized mice and by intra-arterial recording under anesthesia during clearance studies in COX-1 gene–deficient (COX-1−/−) and wild (COX-1+/+) mice. The second aim of this study was to evaluate the role of COX-1 on the circadian regulation of several bioactive markers. Mice urine samples were collected separately during day and night. The renal excretion of sodium, potassium, metanephrine, normetanephrine, aldosterone, nitrate plus nitrite (NOx), and prostaglandins or their metabolites (TxB2, 6-ketoPGF1α, PGE2, and 11-βPGF2α) were measured to assess their potential roles in the functional changes observed.

Methods

Animals

The generation and genotyping of COX-1 gene–deficient mice (Black6/129 background) have been reported.21 Male homozygous (COX-1−/−) and wild (COX-1+/+) mice were housed in a quiet room at 25°C with a 12-hour light/dark cycle with free access to food and water. This study was approved by the Georgetown University Animal Care and Use Committee.

Telemetry

The surgical procedure to implant telemetry is detailed in the online data supplement (available at http://www.hypertensionaha.org). The hourly BP, heart rate (HR), and physical activity data were collected, and the mean and standard error of the mean (SEM) values for groups of mice in synchronized time were calculated and plotted as shown in Figure 1. The mean of the lowest 4 sets of MAPs, HRs, and physical activities during the 6:00 AM to 6:00 PM (day time) within the same day (sleep period) and the highest 4 sets of MAPs, HRs, and physical activities during the next 6:00 PM to 6:00 AM (nighttime: awake time period) were calculated in individual COX-1−/− (n=10) and COX-1+/+ mice (n=9).
Renal Excretion of Sodium, Potassium, TxB2, 6-ketoPGF1α, PGE2, 11-βPGF2α, Nephrines, and NOx

Mice (n=6 each group) were housed in mouse metabolic cages (Nalgene Nunc International, Rochester, NY) with NOx-free synthetic diet (Na content 0.4 g/100 g). Urine was collected from 6:00 AM to 6:00 PM for sleep (daytime) sample and the next 6:00 PM to 6:00 AM for awake (nighttime) sample. Measurement of each parameter is detailed in the online data supplement.

Chemical Methods and Statistics

Chemical methods were those published previously.22 Statistics are detailed in the online data supplement.

Results

Hematocrit, Total Body and Kidney Weights, Plasma Electrolytes, and Urinary Volume of COX-1−/− and COX-1+/+ Mice

There were no differences in these parameters between COX-1+/+ and COX-1−/− mice (supplemental Table I, available online at http://www.hypertensionaha.org).

MAP, HR, and Physical Activity Measured by Telemetry

Figure 1 shows the hourly mean±SEM values of MAP (Figure 1A), HR (Figure 1B), and physical activity (supplemental Figure I) in groups of COX-1−/− and COX-1+/+ mice measured by telemetry for 5 days. In both groups, MAP, HR, and activity were elevated at night (awake) and reduced during the day (sleep). Figure 2 shows the calculated sleep and awake MAPs (Figure 2A) and sleep-to-awake BP ratios (Figure 2B). Although there was no significant difference in awake MAP (COX-1+/+: 131±2 mm Hg; COX-1−/−: 126±3 mm Hg), the sleep MAP (COX-1+/+: 93±1 mm Hg; COX-1−/−: 97±2 mm Hg) and the sleep-to-awake BP ratio was higher in COX-1−/− mice by 8.6%. This was accompanied by a 5.7% increase in sleep-to-awake HR ratio (Figure 3), but there were no differences in physical activity (supplemental Figure II).

MAP and HR Under Anesthesia

When measured by direct intra-arterial catheterization under anesthesia with moderate salt loading, the MAP of COX-1−/− mice (124±4 mm Hg) was higher than COX-1+/+ mice (109±5 mm Hg), but there was no difference in HR (supplemental Figure III).
Renal Function Under Anesthesia

The glomerular filtration rate was not different (COX-1−/−: 0.89±0.06 mL·min⁻¹·g⁻¹; COX-1+/+: 0.93±0.04 mL·min⁻¹·g⁻¹), but COX-1−/− mice had a reduced renal blood flow (RBF) (COX-1−/−: 4.7±0.2 mL·min⁻¹·g⁻¹; COX-1+/+: 4.1±0.2 mL·min⁻¹·g⁻¹; supplemental Figure IVA and IVB) and increased filtration fraction (FF) (COX-1−/−: 34±2%; COX-1+/+: 40±2%) and renal vascular resistance (RVR) (COX-1−/−: 24±2 mm Hg·mL⁻¹·min⁻¹·g⁻¹; COX-1+/+: 31±2 mm Hg·mL⁻¹·min⁻¹·g⁻¹; supplemental Figure IVC and IVD).

Renal Excretion of Sodium and Potassium

The excretion of sodium and potassium was reduced during sleep (supplemental Figure V). COX-1 did not affect excretion of sodium or potassium.

Renal Excretion of TxB₂, PGE₂, 6-ketoPGF₁₀α, and 11-βPGF₂α

The renal excretion of TxB₂ in the COX-1+/+, awake, COX-1−/−, awake, COX-1+/+, sleep, and COX-1−/−, sleep groups are 4.0±0.2, 0.4±0.1, 3.8±0.3, and 0.4±0.1 g·g⁻¹·creatinine·10⁻⁶, respectively. The renal excretion of PGE₂ in the COX-1+/+, awake, COX-1−/−, awake, COX-1+/+, sleep, and COX-1−/−, sleep groups are 1.9±0.4, 0.4±0.1, 1.4±0.6, and 0.4±0.1 g·g⁻¹·creatinine·10⁻⁶, respectively. The renal excretion of 6-ketoPGF₁₀α in the COX-1+/+, awake, COX-1−/−, awake, COX-1+/+, sleep, and COX-1−/−, sleep groups are 1.6±0.1, 1.0±0.1, 1.3±0.1, and 0.8±0.1 g·g⁻¹·creatinine·10⁻⁶, respectively. The renal excretion of 11-βPGF₂α in the COX-1+/+, awake, COX-1−/−, awake, COX-1+/+, sleep, and COX-1−/−, sleep groups are 0.09±0.009, 0.06±0.004, 0.06±0.005, and 0.05±0.003 g·g⁻¹·creatinine·10⁻⁶, re-

![Graphs showing renal excretion of TxB₂, PGE₂, 6-ketoPGF₁₀α, and 11-βPGF₂α.](http://hyper.ahajournals.org/Downloaded from http://hyper.ahajournals.org)

**Figure 4.** Mean±SEM values for renal excretion of TxB₂ (A), PGE₂ (B), 6-ketoPGF₁₀α (C), and 11-βPGF₂α (D).
respectively. COX-1<sup>−/−</sup> had a 89% reduction in the excretion of TxB<sub>2</sub>, a 76% reduction in PGE<sub>2</sub>, a 40% reduction in 6-ketoPGF<sub>1α</sub>, and a 27% reduction in 11β-PGF<sub>2α</sub>. Excretion of 6-ketoPGF<sub>1α</sub> and 11β-PGF<sub>2α</sub> were reduced during sleep, but TxB<sub>2</sub> or PGE<sub>2</sub> were not changed (Figure 4).

Renal Excretion of Catecholamine Metabolites

The renal excretion of metanephrine in the COX-1<sup>−/−</sup> awake, COX-1<sup>−/−</sup> sleep, and COX-1<sup>+/+</sup> sleep groups are 0.037±0.005, 0.049±0.004, and 0.032±0.004, respectively. The renal excretion of normetanephrine in the COX-1<sup>−/−</sup> awake, COX-1<sup>−/−</sup> sleep, and COX-1<sup>+/+</sup> sleep groups are 6.9±0.9, 5.1±0.9, and 3.2±0.6, respectively. Excretion of metanephrine was not different during awake time and sleep time but was increased in COX-1<sup>−/−</sup> mice. Excretion of normetanephrine was reduced during sleep time. COX-1<sup>−/−</sup> mice had a higher normetanephrine excretion only during the sleep time (Figure 5A and 5B).

Renal Excretion of NOx and Aldosterone

The renal excretion of NOx in the COX-1<sup>−/−</sup> awake, COX-1<sup>−/−</sup> sleep, and COX-1<sup>+/+</sup> sleep groups are 0.33±0.06, 0.22±0.04, and 0.12±0.02 mol·mol<sup>−1</sup>·creatinine·10<sup>−3</sup>, respectively. The renal excretion of aldosterone in the COX-1<sup>−/−</sup> awake, COX-1<sup>−/−</sup> sleep, and COX-1<sup>+/+</sup> sleep groups are 0.017±0.002, 0.017±0.003, and 0.010±0.002 g·g<sup>−1</sup>·creatinine·10<sup>−3</sup>, respectively. Excretion of NOx and aldosterone were reduced during sleep time. COX-1<sup>−/−</sup> mice had reduced excretion of NOx but not aldosterone (Figure 5C and 5D).

Correlation Analysis Among Tx/PGs Metabolites and NOx

Renal excretion of NOx was positively correlate with excretion of 6-ketoPGF<sub>1α</sub> and 11β-PGF<sub>2α</sub> (Figure 6A and 6B), but not with TxB<sub>2</sub> or PGE<sub>2</sub> (supplemental Figure VI).
Correlation Analysis Between Sodium and NOx
Renal excretion of sodium was positively correlated with NOx (Figure 6C).

Discussion
A novel finding is that COX-1/−/− mice have higher sleep time MAP and increased ratios of sleep:awake BP and HR. The importance of circadian rhythm in BP was recognized by O’Brien et al who introduced the “dipper/nondipper” classification and showed that nondipper status is a risk factor for stroke. Staessen et al and Okubo et al15,16 showed further that a 5% increment in the night-to-day BP ratio is associated with a 20% increase in cardiovascular events or death even in normotensive subjects. The normal decrement in night-to-day BP in humans has been related to nocturnal reductions in physical activity18 and sympathetic nervous system tone. However, increased NaCl reabsorption19,20 or endothelial dysfunction21 impair the normal night-to-day BP ratio. We found that COX-1 deficiency does not change physical activity, but increases sleep:awake HR ratio. Because HR is regulated by the sympathetic nervous system, a blunting of the nocturnal reduction in the sympathetic nervous system tone could be a cause for the higher sleep:awake BP and HR ratios in COX-1/−/− mice. We measured normetanephrine excretion to index sympathetic nervous system activity. The result confirmed that normetanephrine excretion in wild-type mice declines by 60% while the animals are asleep. In contrast, there were no circadian changes in COX-1/−/− mice (Figure 5B) (interaction day−night×COX-1; P<0.01). Consequently, COX-1/−/−, relative to COX-1+/+, mice have an increased normetanephrine excretion while asleep, consistent with increased sympathetic nervous system tone. COX-1/−/− mice also had increased excretion of metanephrine, suggesting increased epinephrine secretion. The excretion of NOx or aldosterone failed to explain the higher sleep:awake BP ratio in COX-1/−/− mice (Figure 5C and 5D) (interaction day−night×COX-1; not significant).

Presently, the cause of the enhanced sympathetic nervous system activity during sleep time in COX-1/−/− mice is not clear. PGE2 can activate sympathetic nerves in kidney23 and in central nervous system (paraventricular nucleus of hypothalamus),24 and PGD2 can activate norepinephric neurons in the hypothalamus. Whether local COX-1–dependent PGE2/D2 production in the brain or peripheral nerves underlies the circadian rhythm of sympathetic nervous system activity requires further study.

COX-1/−/− mice had an increased MAP when studied under anesthesia with moderate saltwater loading (1.5% albumin in saline at a rate of 0.35 mL/h) accompanied by an increased RVR and FF and a decreased RBF. This renal vasoconstriction may have contributed to the increased BP. Because anesthetic drugs blunt the action of catecholamines, the cause

Figure 6. Correlation analysis among renal excretion of NOx and 6-ketoPGF1α (A), 11-βPGF2α (B), and sodium (C).
of the renal vasoconstriction in COX-1−/− mice under anesthesia is not likely to be attributed to an increase in catecholamines and sympathetic nervous activity. It also cannot be attributed to an increased ratio of vasoconstrictor TxA2 to vasodilator PGs, because COX-1−/− mice had a more marked reduction in renal excretion of TxB2 than 6-ketoPGF1α, consistent with previous studies that TxA2 is mainly generated by COX-1, whereas both COX-1 and COX-2 contribute to the production of PGH2.26 There was no difference in excretion of aldosterone between COX-1−/− and COX-1+/+ mice.

Our finding that COX-1−/− mice have a reduced excretion of NO metabolites suggests that the cause for the renal vasoconstriction seen in COX-1−/− mice may rather relate to defective generation of NO. Similar to our finding in COX-1−/− mice, NSAIDs reduce NO production.27,28 We found a strong positive correlation between the renal excretion of sodium and NOx. Inhibition of NO generation with l-nitroethyl arginine ester causes salt-sensitivity, accompanied by reduced renal Na+ excretion and renal vasoconstriction with an elevated FF and hypertension.29–33 These are all features of the COX-1−/− mouse in this study. Therefore, a reduction in NO generation may underlie the hemodynamic change seen in COX-1−/− mice.

The role of PGs in NO production needs further study. We found strong correlations between the renal excretion of NOx with PG1 and PGD2 metabolites, but not with TxA2 or PGE2 metabolites. Zenge24 has shown PG12-induced pulmonary vasodilation is mediated by NO release. Niwano25 has shown that the PG12 analogue, Beraprost sodium, increases endothelial nitric oxide synthase expression and NO from endothelial cells via cAMP signaling. PGD2 increases endothelial nitric oxide synthase expression and NO generation in the developing choroids.36 The PGD2 metabolite, 15-deoxy-e12,14-PGJ2 (15d-PGJ2), also increases NO generation by endothelial cells,37 but the molecular mechanism is not yet established.

Perspectives
NSAIDs can cause renal vasoconstriction and raise BP in patients with hypertension. The similar profile of effects of COX-1 gene deletion in this study to that of NSAIDs recipients suggest that some of the adverse effects associated with NSAIDs use may originate from inhibition of COX-1. Likely, these potential adverse cardiovascular effects are offset by inhibition of platelet COX-1 that will inhibit thrombogenesis. The profound reduction in NOx excretion and increased nocturnal excretion of normetanephrine in COX-1−/− mice implies a new role for this enzyme, and likely its products, PG12 and PGD2 or their metabolites in NO generation and sympathetic drive. Defects in NO generation are linked to atherosclerosis and adverse cardiovascular outcome in many clinical studies. These findings expose complex, and perhaps discordant, effects of COX-1 metabolites on potential cardiovascular risk factors that add to the growing concern of unanticipated cardiovascular events in patients using drugs that inhibit cyclooxygenase.

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References
3. Johnson AG, Nguyen TV, Day RO. Renal excretion of NOx and nitrite, 15-deoxy-delta12,14-PG J2 (15d-PGJ2), also increases NO generation by endothelial cells,37 but the molecular mechanism is not yet established.

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Cyclooxygenase-1–Deficient Mice Have High Sleep-to-Wake Blood Pressure Ratios and Renal Vasoconstriction

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