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<tr>
<td>Citation</td>
<td>Antioxidants &amp; Redox Signaling, 2012, v. 16 n. 4, p. 363-373</td>
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<td>Issued Date</td>
<td>2012</td>
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<td>URL</td>
<td><a href="http://hdl.handle.net/10722/146896">http://hdl.handle.net/10722/146896</a></td>
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<td>Rights</td>
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Oxidative Stress-Dependent Cyclooxygenase-2-Derived Prostaglandin F$_{2\alpha}$ Impairs Endothelial Function in Renovascular Hypertensive Rats

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Abstract

**Aims:** The role of endothelium-derived contracting factors (EDCFs) in regulating renovascular function is yet to be elucidated in renovascular hypertension (RH). The current study investigated whether oxidative stress-dependent cyclooxygenase (COX)-2-derived prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) impairs endothelial function in renal arteries of renovascular hypertensive rats (RHR). **Results:** Renal hypertension was induced in rats by renal artery stenosis of both kidneys using the 2-kidney 2-clip model. Acute treatment with reactive oxygen species (ROS) scavengers, COX-2 inhibitors, and thromboxane-prostanoid receptor antagonists, but not COX-1 inhibitors, improved endothelium-dependent relaxations and eliminated endothelium-dependent contractions in RHR renal arteries. Five weeks of treatment with celecoxib or tempol reduced blood pressure, increased renal blood flow, and restored endothelial function in RHRs. Increased ROS production in RHR arteries was inhibited by ROS scavengers, but unaffected by COX-2 inhibitors; whereas increased PGF$_{2\alpha}$ release was reduced by both ROS scavengers and COX-2 inhibitors. ROS also induced COX-2-dependent contraction in RHR renal arteries, which was accompanied by the release of COX-2-derived PGF$_{2\alpha}$. Further, chronic tempol treatment reduced COX-2 and BMP4 upregulation, p38MAPK phosphorylation, and the nitrotyrosine level in RHR renal arteries. **Conclusion:** These findings demonstrate the functional importance of oxidative stress, which serves as an initiator of increased COX-2 activity, and that COX-2-derived PGF$_{2\alpha}$ plays an important role in mediating endothelial dysfunction in RH. **Innovation:** The current study, thus, suggests that drugs targeting oxidative stress-dependent COX-2-derived PGF$_{2\alpha}$ may be useful in the prevention and management of RH. *Antioxid. Redox Signal.* 16, 363–373.

Introduction

Renal artery stenosis is the major cause of renovascular hypertension (RH) and can lead to reduced renal blood flow and end-stage renal damage (36). The role of renal vasculature in RH was established by Goldblatt et al. (15), who demonstrated that partial obstruction of the renal artery increased mean arterial pressure, which has the features of RH in humans. The mechanisms responsible for the development of RH remain largely undefined, but oxidative stress plays an important role (12, 26). Nevertheless, it is unclear how oxidative stress affects renovascular function in RH.

**Innovation**

Endothelium-derived contracting factors (EDCFs) participate in the development and maintenance of hypertension. This research reported the functional importance of cyclooxygenase (COX)-2 derived prostaglandin F$_{2\alpha}$ in response to oxidative stress as the major EDCF to impair endothelium-dependent relaxation and enhance endothelium-dependent contraction in renal arteries of renal hypertension induced by renal artery stenosis. Antioxidant or COX-2 inhibition can reduce blood pressure and improve endothelial function in renal hypertensive rats.
Endothelial cell function reflects the condition of vascular health and predicts the severity of future vascular complications (37). Impaired endothelium-dependent relaxations (EDRs) have been reported in aortae (4, 28), superior mesenteric arteries (35), and mesenteric resistance arteries (7, 10) in renovascular hypertensive rats (RHRs). However, little is known about the impact of RH on vascular reactivity of renal arteries and renal blood flow. The mechanisms responsible for reduced EDRs may include activation of the renin-angiotensin-aldosterone system (20, 26), and overproduction of superoxide derived from the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (19, 21, 23). In contrast, no study has been directed toward exploring the possible effect of RH on endothelium-dependent contractions (EDCs). The endothelium-derived contracting factors (EDCFs) that contribute to endothelial dysfunction are reported to be either cyclooxygenase (COX)-mediated arachidonic acid-derived prostaglandins (2, 13) or reactive oxygen species (ROS) (44), which act on vascular smooth muscle to cause contractions.

Earlier studies showed that selective COX-1 inhibitors prevent acetylcholine-induced EDCs in aortae of spontaneously hypertensive rats (SHRs) (44) and improve EDRs in mesenteric arteries in angiotensin II-infused mice (39), thus suggesting that COX-1-derived EDCFs impair endothelial function. More recently, COX-2-derived constricting prostanooids have been suggested to attenuate EDRs in SHR mesenteric arteries (40). Indeed, COX-2 inhibitors improve endothelial function in patients with hypertension and coronary heart disease (5, 41). Nevertheless, whether COX-derived prostaglandins contribute to endothelial dysfunction in RH has not been examined.

Although COX-derived prostaglandins and ROS are suggested to be EDCFds, it remains unclear whether ROS can stimulate endothelial COX isoforms to release EDCFds in renal arteries of RHRs. The role of EDCFds in regulating renovascular function and renal blood flow in vivo is yet to be elucidated in RH. Therefore, the current study aims at determining whether ROS regulate COX-derived prostaglandins to cause endothelial dysfunction in RH.

Results

COX-2 mediates impairments in EDRs in renal arteries of RHRs

Acetylcholine-induced EDRs were blunted in renal arteries from RHRs 5 weeks after the induction of renal artery stenosis and further reduced 10 weeks after surgery compared with those from sham-operated rats (Fig. 1A, B). Renal arteries from RHRs exhibited a biphasic response to acetylcholine, with an initial relaxation followed by a contraction at concentrations of acetylcholine higher than 1 μM (Fig. 1A, B). Endothelium-independent relaxations in response to sodium nitroprusside (SNP) (Fig. 1C) or to nitroglycerin (Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/ars) were similar between the two groups. Thirty minutes of treatment with RHR renal arteries with the nonselective COX inhibitor indomethacin (Fig. 1E), COX-2 inhibitors celecoxib, NS398, DuP697, or the thromboxane prostanoid (TP) receptor antagonist S18886 restored the impaired EDRs (Fig. 1D, F); whereas COX-1 inhibitors SC-560 and valeryl salicylate (VAS) had no effect (Fig. 1E). The protein synthesis inhibitor cycloheximide did not modify EDRs in renal arteries (Fig. 1G).

COX-2 mediates EDCs in renal arteries of RHRs

Acetylcholine elicited EDCs in renal arteries from RHRs (5 and 10 weeks after surgery) compared with those from control rats (Fig. 2A, B). Acetylcholine-induced EDCs were only observed in the presence of N\(^-\)nitro-L-arginine methyl ester (L-NNAME) in rings with endothelium (Supplementary Fig. S2). Indomethacin (Fig. 2C), celecoxib, DuP697 or NS398 (Fig. 2C), or S18886 (Fig. 2D) abolished EDCs; whereas SC-560, VAS (Fig. 2E), or cycloheximide (Fig. 2F) had no effect.

Chronic celecoxib or tempol treatment increases renal blood flow, reduces blood pressure, and improves endothelial function in RHRs

Renal blood flow was measured by magnetic resonance image (MRI) in the renal cortex, which was selected as the region of interest for the intensity analysis of Gd-DOTA-induced signal enhancement. The maximum intensity was observed in the aorta first, followed by the renal cortex ~20 s after bolus injection of Gd-DOTA into the tail vein, then the outer and inner medulla, and finally, the renal pelvis. Renal blood flow assessed by cortical signal enhancement (Fig. 3A) was reduced in RHRs compared with control rats. The reduced flow was restored after treatment with celecoxib or tempol (Fig. 3B, C), accompanied by a reduction in systolic blood pressure in RHRs; whereas celecoxib or tempol treatment did not affect the blood pressure of control rats (Fig. 3D). After drug treatment, EDRs of RHR renal arteries were improved (Fig. 3E), and EDCs were reduced (Fig. 3F). Acute exposure to the putative NADPH oxidase inhibitor apocynin improved EDRs in RHR renal arteries but did not cause further improvement in celecoxib-treated RHRs (Supplementary Fig. S3).

ROS trigger COX-2-dependent endothelial dysfunction in renal arteries of RHRs

Tiron plus DETCA, or tempol (ROS scavengers) augmented EDRs (Fig. 4A) and abolished EDCs (Fig. 4B) in renal arteries from RHRs, which were unaffected by the xanthine oxidase inhibitor, allopurinol (Supplementary Fig. S4). Electron paramagnetic resonance (EPR) spectra showed an increased generation of superoxide anions (O\(_2^-\)) in RHR renal arteries on acetylcholine stimulation in the presence of L-NNAME (Fig. 4C, D). Renal arteries from tempol-treated RHRs but not from celecoxib-treated RHRs showed a reduction of ROS generation (Fig. 4C, D). Further, H\(_2\)O\(_2\) (Fig. 4E) and hypoxanthine + xanthine oxidase (HX-XO)-induced contractions (Fig. 4F) in RHR renal arteries (3.43 ± 0.56 mN/mm for H\(_2\)O\(_2\) and 2.87 ± 0.66 mN/mm for HX-XO) were greater than those of control rats (1.09 ± 0.13 mN/mm for H\(_2\)O\(_2\) and 0.11 ± 0.03 mN/mm for HX-XO). Both Celecoxib and S18886 prevented H\(_2\)O\(_2\)- or HX-XO-induced responses; whereas SC-560 did not (Fig. 4E, F). Removal of the endothelium reduced contractions elicited by H\(_2\)O\(_2\) or HX-XO increased COX-2 expression in renal arteries and in primary aortic endothelial cells from control rats (Supplementary Fig. S5).
COX-2-derived prostaglandin F<sub>2α</sub> mediates endothelial dysfunction in renal arteries of RHRs

The amount of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) (Fig. 5A), 8-isoprostane (Fig. 5B), 6-keto PGF<sub>1α</sub> (metabolites of prostacyclin [PGI<sub>2</sub>], Fig. 5C), PGE<sub>2</sub> (Supplementary Fig. 5A), PGD<sub>2</sub> (Supplementary Fig. 5B), and TXB<sub>2</sub> (metabolites of TXA<sub>2</sub>, Supplementary Fig. 5C) released in response to acetylcholine (100 μM) stimulation was greater in RHR than in control rat renal arteries. Chronic treatment with celecoxib or tempol inhibited acetylcholine-stimulated release of PGF<sub>2α</sub>, 8-isoprostane, PGE<sub>2</sub>, and PGD<sub>2</sub> but not 6-keto PGF<sub>1α</sub> and TXB<sub>2</sub> (Fig. 5A–C and Supplementary Fig. S5). A 30-min exposure to celecoxib or tiron plus DETCA inhibited the acetylcholine-stimulated release of all six prostaglandins (Fig. 5A–C and Supplementary Fig. S6), whereas SC-560 reduced PGE<sub>2</sub>, PGD<sub>2</sub>, and TXB<sub>2</sub> (Supplementary Fig. S5) without affecting PGF<sub>2α</sub>, 8-isoprostane, and 6-keto PGF<sub>1α</sub>.

PGF<sub>2α</sub> and 8-isoprostane produced greater contractions in renal arteries of RHRs than in control arteries (Fig. 5D, E), whereas PGI<sub>2</sub> produced comparable responses in both groups (Fig. 5F). When PGF<sub>2α</sub> was administered to RHR renal artery preparations at a concentration (~38 nM) comparable to the value measured by enzyme immunoassay (EIA) in acetylcholine-stimulated RHR renal arteries, the induced contractions were similar in magnitude to EDCs (Fig. 5D). In contrast, the EIA-detected amounts of 8-isoprostane at 0.38 μM did not cause contraction (Fig. 5E, F). In addition, H<sub>2</sub>O<sub>2</sub> at 100 μM increased the release of PGF<sub>2α</sub> from RHR renal arteries, which was reduced by celecoxib but not SC-560 (Fig. 5G). Plasma PGF<sub>2α</sub> concentration was also decreased after chronic celecoxib or tempol administration to RHRs (Fig. 5H). Further, a 30-min exposure to 7 nM PGF<sub>2α</sub> reduced EDRs in renal arteries from control rats, which was prevented by pretreatment with S18886 (0.1 μM) but not with celecoxib (3 μM) (Fig. 5I).

Increased expression of COX-2 and oxidative stress biomarker proteins in renal arteries of RHRs

The levels of 3-nitrotyrosine (Fig. 6A), COX-2 (Fig. 6B), BMP4 (Fig. 6C), and the phosphorylated form of p38MAPK (Fig. 6D) were significantly increased in renal arteries of RHRs compared with those from control rats. Multiple bands of
nitrotyrosine at ~60, 55, 40, and 35 kDa were detected in renal arteries (Fig. 6A). Chronic treatment with tempol but not celecoxib inhibited the up-regulation of 3-nitrotyrosine, COX-2, BMP4, and p38 phosphorylation in RHR renal arteries. In contrast, the protein levels of COX-1 (Fig. 6B) and NOX2 (Fig. 6F) were similar among all groups. The expression of p67phox (Fig. 6E) was increased in renal arteries from RHR but remained unaffected after celecoxib or tempol treatment (Fig. 6E).

Discussion

The current study provides evidence for a crucial role of ROS-dependent COX-2-derived PGF2α in endothelial dysfunction in RH. The major findings are as follows: (i) the impaired EDRs and the augmented EDCs in renal arteries of RHRs are restored by treatment with COX-2 inhibitors, TP receptor antagonists and ROS scavengers; (ii) chronic treatment with celecoxib or tempol increases renal blood flow, reduces blood pressure, and improves endothelial function in RHRs; and (iii) ROS are a prerequisite for the occurrence of endothelial dysfunction through the generation of COX-2-derived PGF2α, suggesting that ROS activates COX-2 to release PGF2α, which impairs EDRs and induces EDCs in RHR renal arteries.

ROS contribute to the development of endothelial dysfunction in renal vasculature during the development of RH. We showed that reduced EDRs in RHR renal arteries were reversed by ROS scavengers, under both in vitro and in vivo conditions. In addition, the p67phox level increased, and NADPH oxidase inhibitor apocynin improved EDRs in RHR renal arteries (Supplementary Fig. S2). ROS production from NADPH oxidase is a major source of oxidative stress in endothelial dysfunction associated with hypertension (17, 18, 23, 30). In aortae of renovascular hypertensive mice, the blunted EDRs were prevented by genetic deletion of the NADPH oxidase subunit gp91phox (23). Recent studies also demonstrated that antioxidant treatment is able to lower blood pressure and improve EDRs in RHRs (4). The current results show that tempol treatment improved EDRs in renal arteries, reduced blood pressure (Supplementary Fig. S7), and increased renal blood flow in vivo in RHRs.

An earlier report indicated that nonselective COX inhibition normalizes the blunted EDRs in small mesenteric arteries in RHRs (10). To the best of our knowledge, there is no report about the potential impact of RH on EDC responses. The current study demonstrates that COX-2, rather than COX-1, is the key enzyme mediating the effect that reduces EDRs and augments EDC in RHR renal arteries. In addition, up-regulation of COX-2 rather than COX-1 was observed in RHR renal arteries. It was reported that COX-1, rather than COX-2, mediates EDCs in aortae from SHR (45). However, COX-2-derived constricting prostanoids have been recently suggested to impair EDRs in mesenteric arteries of SHR (40). Taken together, these observations suggest that there could be a significant difference in the involvement of COX isoforms depending on the etiology of hypertension and/or the specific vascular bed chosen for examination.
ROS might function as EDCFs in the canine basilar artery (25), and ROS facilitated EDC in aortae of SHR (45). In addition, COX-derived prostaglandins were suggested to be one major source of EDCF apart from superoxide anions (1, 14, 39, 46). The current study indicates that EDCFs derived from COX-2 were released in response to ROS stimulation based on the following findings. First, ROS scavengers prevented EDCs. Second, acetylcholine-stimulated ROS production was greater in RHR renal arteries and ROS-generating agents, such as H$_2$O$_2$ and HX-XO, could elicit celecoxib-inhibitable contractions and upregulate COX-2 expression. Moreover, the increase in peroxynitrite-induced protein nitration (48) observed in RHR renal arteries was inhibited after tempol treatment but was unaffected by celecoxib. On the basis of our previous report, it is worth noting that ROS is also an upstream regulator of COX-2 expression and activity that induces endothelial dysfunction in mouse arteries (42). The current study shows that BMP4, an upstream mediator of endothelial dysfunction, was upregulated in RHR renal arteries, which is consistent with our previous finding of increased BMP4 expression in renal arteries from patients with hypertension and SHRs (42). Of importance, treatment with the antioxidant tempol but not celecoxib inhibited BMP4 upregulation. Although BMP4 is an upstream regulator of the expression of pro-inflammatory genes in endothelial cells in response to oxidative stress (29, 31, 33), BMP4 expression can

![Image](image-url)
also be regulated by oxidative stress (6, 33). We also found that p38 phosphorylation in RHR renal arteries was reduced after tempol treatment, thus suggesting that p38 was involved in ROS-induced endothelial dysfunction in RHRs (42).

The current study extends the understanding of arachidonic acid derivatives as EDCFs, which was supported by measurements of prostaglandin release by EIA and use of exogenously applied prostaglandins to induce vasoconstriction. The EIA results showed increased release of all types of prostaglandins in RHR renal arteries in response to acetylcholine, whereas only PGF2α, 6-keto PGF1α (the stable metabolite of PGI2), and 8-isoprostane were indicated to be COX-2-derived EDCF candidates. To verify this, exogenous PGI2, PGF2α, or 8-isoprostane were applied to determine whether they caused contractions in renal arteries. Only PGF2α produced contraction at a concentration near to that determined by EIA upon acetylcholine stimulation. Although 8-isoprostane and PGI2 contracted renal arteries, the threshold concentration for both prostaglandins to evoke contraction was much higher than that detected by EIA in response to acetylcholine. The involvement of PGD2, PGE2, or TXA2 was unlikely, because their release was prevented by COX-1 inhibitors that did not block EDCs. These results support the conclusion that PGF2α is the most likely EDCF in RHR renal arteries. Previous studies on oxidative stress-dependent COX-2 induction were mainly focused on endothelial cell inflammation and apoptosis, and PGE2 was considered the major contributor to the effect of COX-2 induction (3, 8, 24, 34). However, PGF2α was less studied (3, 22). The current study provides new information about the critical role of COX-2 and PGF2α in renal vascular hypertension. It is also noted that the pathological role of PGF2α has been recently revealed, as this prostaglandin increases blood pressure and promotes atherosclerosis in mice (47). The current results also show that COX-2 inhibition, either in vivo or ex vivo, can reduce the production of the PGI2 metabolite 6-keto-PGF1α, which is unaffected by COX-1 inhibitors, thus suggesting that COX-2 is the major enzyme responsible for PGI2 synthesis in renal vasculature. This finding is different from that of a previous study concluding that COX-1 is the major enzyme of PGI2 synthesis (9). The relative importance of COX-2-derived PGI2 in the development of renal hypertension is yet to be examined.

The current study also shows that ROS do not cause contraction directly in RHR renal arteries. A more likely explanation is that the increased production of ROS stimulates endothelial COX-2 to release PGF2α, which activates TP receptors to cause contraction of vascular smooth muscle cells under renovascular hypertensive conditions. This was supported by the following observations: (i) Exogenous ROS (H2O2 or HX-XO) produced greater contractions in renal arteries from RHRs than control; (ii) ROS-induced contractions were abolished by celecoxib and S18886; (iii) ROS-induced contractions were reduced in arteries without endothelium; (iv) H2O2 stimulated PGF2α release was prevented by celecoxib; (v) The acetylcholine-stimulated release of PGF2α was inhibited by ROS scavengers in RHR arteries; (vi) PGF2α attenuated EDRs in renal arteries from control rats, which was prevented by S18886 but not by a COX-2 inhibitor. Moreover, chronic treatment with tempol, but not celecoxib, attenuated the up-regulation of both nitrotyrosine and COX-2 in RHR.

![FIG. 4. Reactive oxygen species (ROS) triggers COX-2-dependent endothelial dysfunction in RHRs. Effects of 30-min exposure to tiron (1 mM) plus DETCA (100 μM), or tempol (100 μM) on EDRs (A) and EDCs (B) in RHR renal arteries. (C) Electron paramagnetic resonance (EPR) spectroscopy showing markedly increased amplitude of ROS signal in response to ACh (100 μM) in RHR renal arteries. (D) Summarized data for EPR signal intensity. Effects of SC-560 (0.3 μM), celecoxib (3 μM), S18886 (0.1 μM), and endothelium removal on contractions evoked by H2O2 (100 μM) (E) and HX-XO (100 μM hypoxanthine plus 0.01 units/ml xanthine oxidase (F) in RHR renal arteries. Results are mean±SEM of four to six experiments. *p<0.05 versus control °p<0.05 versus RHR. –E, without endothelium.](image-url)
arteries. Taken together, the current results support the role of ROS as a trigger in the acetylcholine-stimulated release of endothelial COX-2-derived PGF$_{2\alpha}$ in RHR renal arteries. However, there are other potential factors that might also be involved in the enhanced contraction in RHR renal arteries including diminished nitric oxide (NO) production, oxidative stress per se, and angiotensin II (11, 32).

In summary, the current study demonstrates that both celecoxib and tempol reduce blood pressure, increase renal blood flow, and improve endothelial function in RHRs. In RHR renal arteries, ROS is the initiator that activates endothelial COX-2 to release PGF$_{2\alpha}$, the most likely EDCF to participate in endothelial dysfunction in RHRs. Drugs targeting this ROS-COX-TP cascade may be useful in the prevention and management of RH.

**Materials and Methods**

**Chemicals**

Cycloheximide was purchased from Calbiochem, EMD Biosciences. VAS, PGF$_{2\alpha}$, and PGI$_2$ were from Cayman Chemical. Indomethacin, 5-bromo-2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-1H-thiophene (DuP-697) and N-[2-(cyclohexyllox)-4-nitrophenyl]-methanesulfonamide (NS-398), indomethacin, and 8-isoprostane were from Tocris (Avonmouth). SC-560 and 3-[6-amino-(4-chlorobenzensulphonyl)-2-methyl-5,6,7,8-tetrahydro napht-1-yl]propionic acid (S18886) were kind gifts from Institut de Recherches Servier. Nitroglycerin was from Schwarz Pharma. All others were purchased from Sigma-Aldrich Chemical Co. Acetylcholine, L-NAME, phenylephrine, SNP, nitroglycerin, PGI$_2$, tiron, and tempol were prepared in distilled water; all other drugs were dissolved in dimethylsulfoxide (Sigma-Aldrich).

**Induction of RH and drug treatment**

The experimental protocol approved by the institutional animal care and use committee was consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. RH was induced by a surgical bilateral renal artery stenosis procedure in male Sprague-Dawley rats. The rats (80–100 g) were anaesthetized with ketamine (35 mg kg$^{-1}$) plus xylazine (7 mg kg$^{-1}$). After performing a midline laparotomy, renal arteries of both kidneys were carefully separated from adjacent adhering tissue and then individually occluded by a silver clip (internal diameter: 0.2 mm). Sham-operated rats (Control) were subjected to laparotomy and renal artery separation only. All rats had free access to rat chow and tap water, and were maintained in their cages in a controlled environment at 23°C±1°C with a 12-h dark/light cycle. Five weeks later, RHRs with systolic blood pressure over 180 mmHg were randomly divided into three groups: RHRs receiving vehicle (RHR); RHRs receiving orally administered celecoxib at a dose of 10 mg kg$^{-1}$ day$^{-1}$ for 5 weeks (RHR+Celecoxib); or RHRs receiving tempol (RHR+...
Tempol) at a dose of 10 mg kg\(^{-1}\) day\(^{-1}\) for 5 weeks. Some sham-operated control rats were also orally treated with 10 mg kg\(^{-1}\) day\(^{-1}\) celecoxib (Control + Celecoxib) or 10 mg kg\(^{-1}\) day\(^{-1}\) tempol (Control + Tempol). Systolic blood pressure was measured by a tail-cuff method.

**Vascular reactivity**

Vasoreactivity of the interlobar renal arteries were measured as described (27). The effects of various compounds on EDRs and EDCs were tested. The EDRs occurred in response to the cumulative addition (0.01–30 \(\mu\)M) of acetylcholine in arteries that had been precontracted by phenylephrine (1 \(\mu\)M), and the EDCs were elicited by acetylcholine (0.1–100 \(\mu\)M) after pretreatment with 100 \(\mu\)M L-NAME, which was used to eliminate the interference of endothelium-derived NO. These effects were tested using 30-min incubations with the following compounds: SC-560 (0.3 \(\mu\)M; COX-1 inhibitor); VAS (300 \(\mu\)M; COX-1 inhibitor); celecoxib, NS398, DuP697 (3 \(\mu\)M; COX-2 inhibitor); indomethacin (1 \(\mu\)M; nonselective COX

**FIG. 6. Protein expression in renal arteries of RHRs.** Western blots showing the expression of (A) 3-nitrotyrosine (60, 55, 40, and 35 kDa); (B) COX-2 (72 kDa) and COX-1 (72 kDa); (C) BMP4 (36 kDa); (D) phospho-p38MAPK and total p38MAPK (43 kDa); (E) p67phox (67 kDa); and (F) NOX2 (67 kDa) in renal arteries from control rats, RHRs, and RHRs treated with celecoxib or tempol. GAPDH (37 kDa) was used as a housekeeping protein. Results represent mean ± SEM of four experiments. *\(p<0.05\) versus control, \# \(p<0.05\) versus RHR.
inhibitor), tiron [1 mM; superoxide dismutase mimetic] plus diethyldithiocarbamate acid [DETCA, 100 μM; hydroxyl radical (HO·) scavenger]; tempol (100 μM); apocynin (100 μM, NADPH oxidase inhibitor); allopurinol (100 μM xanthine oxidase inhibitor); S18886 (0.1 μM; thromboxane receptor antagonist); and cycloheximide (10 μM, protein synthesis inhibitor) (45). Endothelium-independent relaxations to SNP or nitroglycerin were studied in arteries without endothelium. Hydrogen peroxide (H2O2, 100 μM), PGF2α, 8-isoprostanate, PGI2, and a combination of hypoxanthine (HX, 100 μM) and xanthine oxidase (XO, 0.01 unit/ml), were used to induce contraction in the presence of 100 μM L-NAME.

**EPR spectroscopy**

The formation of ROS was measured by a reaction with the spin trap agent TEMPO-H using EPR spectroscopy in rat renal arteries. The detailed method is included in the Supplementary Materials.

**Measurement of prostaglandins by EIA**

The amounts of individual prostaglandins were measured with EIA kits (Cayman Chemical) in rat renal arteries. The detailed method is included in the Supplementary Materials.

**Measurement of renal blood flow by MRI acquisition**

MRI studies were performed using a 3 T clinical whole-body imaging system (Achieva; Philips Healthcare) as previously described (16). The detailed method is presented in the Supplementary Materials.

**Protein extraction and western blotting**

Renal arteries were isolated and frozen in liquid nitrogen and homogenized in RIPA lysis buffer. Proteins were extracted from the vessels as previously described (43). Western blot analyses were performed with appropriate specific primary antibodies including COX-2 or COX-1 (1:1000; Cayman Chemical), 3-nitrotyrosine (1:1000; Upstate Biotechnology), BMP4 (1:1000; Sigma), phospho- and total p38MAPK, p67phox (1:1000; Cell Signaling), and NOX2 (1:1000; Abcam).

**Data analysis**

Results are given as mean ± standard error of the mean of n experiments in arteries from different rats. Contractions are expressed as active tension (force recorded/[2 × length of ring]) (38). Western blots were analyzed using Quantity One (Bio-Rad). The protein expression is normalized to the level of GAPDH and is expressed relative to the control. Student’s t-test was used for statistical comparison between two groups, whereas ANOVA was used for comparisons involving more than two groups. The concentration-response curves were analyzed by one-way ANOVA followed by Bonferroni’s post hoc test. Probability values of less than 0.05 indicate statistically significant differences between treatments.

**Sources of Funding**

This study was supported by Hong Kong General Research Fund (465308, 466110, and 465611), UGC Direct Grant (2041450), National Basic Research Program of China (2012CB517805), Focused Investment Scheme from the Chinese University of Hong Kong, and the National Institutes of Health (RC2HL103400, 1U01HL100397).

**Author Disclosure Statement**

No competing financial interests exist.

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Date of first submission to ARS Central, December 29, 2010; date of final revised submission, September 27, 2011; date of acceptance, September 27, 2011.

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**Abbreviations Used**

ACh = acetylcholine

COX = cyclooxygenase

DTPA = diethylenetriaminepentaacetic acid

EDC = endothelium-dependent contraction

EDCF = endothelium-derived contracting factor

EDR = endothelium-dependent relaxation

EIA = enzyme immunoassay

EPR = electron paramagnetic resonance

HX-XO = hypoxanthine+xanthine oxidase

L-NAME = N^6^-nitro-L-arginine methyl ester

NADPH = nicotinamide adenine dinucleotide phosphate

MRI = magnetic resonance image

NO = nitric oxide

PGF\textsubscript{2\alpha} = prostaglandin F\textsubscript{2\alpha}

PGI\textsubscript{2} = prostacyclin

RHR = renovascular hypertensive rat

ROI = region of interest

ROS = reactive oxygen species

SEM = standard error of the mean

SHR = spontaneously hypertensive rat

SNP = sodium nitroprusside

TE = time to echo

TP = thromboxane prostanoid

TR = repetition time

VAS = valeryl salicylate

3D = three-dimensional