A vasoconstrictor role for cyclooxygenase-1-mediated prostacyclin synthesis in mouse renal arteries

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Liu B, Zhang Y, Zhu N, Li H, Luo W, Zhou Y. A vasoconstrictor role for cyclooxygenase-1-mediated prostacyclin synthesis in mouse renal arteries. Am J Physiol Renal Physiol 305: F1315–F1322, 2013—This study was to determine whether prostacyclin [prostaglandin I2 (PGI2)] evokes mouse renal vasoconstriction and, if so, the underlying mechanism(s) and how its synthesis via cyclooxygenase-1 (COX-1) influences local vasomotor reaction. Experiments were performed on vessels from C57BL/6 mice and/or those with COX-1 deficiency (COX-1−/−). Results showed that in renal arteries PGI2 evoked contraction more potently than in carotid arteries, where COX-1 is suggested to mediate prominent endothelium-dependent contraction. A similar result was observed with the thromboxane-prostanoid (TP) receptor agonist U46619. However, in renal arteries TP receptor antagonism, which inhibited the contraction, did not result in any relaxation in response to PGI2. Moreover, we noted that the endothelial muscarinic receptor agonist ACh evoked an increase in the production of the PGI2 metabolite 6-keto-PGF1α, which was prevented by endothelial denudation or COX-1−/−. Interestingly, COX-1−/− was further found to abolish a force development that was sensitive to TP receptor antagonism and result in enhanced relaxation evoked by ACh following NO synthase inhibition. Also, in renal arteries the COX substrate arachidonic acid evoked a vasoconstrictor response, which was again abolished by COX-1−/−. Meanwhile, nonselective COX inhibition did not show any effect in vessels from COX-1−/− mice. Thus, in mouse renal arteries, high expression of TP receptors together with little functional involvement from the vasodilator PGI2 receptors results in a potent vasoconstrictor effect evoked by PGI2. Also, our data imply that endogenous COX-1-mediated PGI2 synthesis leads to vasoconstrictor activity and this could be an integral part of endothelium-derived mechanisms in regulating local renal vascular function.

arachidonic acid; TP receptors; contraction; IP receptors; vasodilation

Cyclooxygenase (COX), which exists in COX-1 and -2 isoforms, metabolizes arachidonic acid (AA) to produce various vasoactive prostanoids. Among them, prostacyclin [prostaglandin I2 (PGI2)] is the major product generated in the endothelium, acting on the PGI2 (IP) receptors on medial smooth muscle to mediate vasodilatation and protect vessels from the development of disease (4, 25, 33). Decreased production of endothelial PGI2 synthesis has been suggested to cause increased incidence of cardiovascular events (8, 12, 40). On the other hand, studies also indicate that the endothelial COX-mediated metabolism generates vasoconstrictor products that act on thromboxane-prostanoid (TP) receptors to mediate vasoconstriction (9, 14, 16, 28, 30, 32, 43). Interestingly, PGI2 can also activate smooth muscle TP receptors (11, 21, 22, 36, 38). This has been explained by an excessive production of PGI2 and/or dysfunction of IP receptors found in conditions such as hypertension (11, 36, 38). Moreover, both TP and IP receptors may concomitantly modulate the vasomotor reaction to PGI2; hence, in vessels with limited functional IP receptors the process of PGI2 synthesis itself is suggested to mediate a vasoconstrictor response even under normal physiological conditions (7, 19, 21, 30).

Renal vascular tone plays an important role in controlling systemic blood pressure and balance of body fluid and salts. In the renal vasculature, PGI2 has been considered as endothelium-derived vasodilator; a decrease in its synthesis is suggested to cause increased renal vascular resistance (12, 15). At the same time, endothelium-derived vasoconstrictor activity has also been found to exist in renal vasculature (5, 13, 14, 23, 39). Moreover, our recent studies suggest that in porcine interlobar renal arteries COX-1-mediated endothelial PGI2 synthesis is responsible for endothelium-dependent vasoconstrictor activity evoked by agonists, such as bradykinin (22). On the other hand, the effect of PGI2 on a given vascular bed may vary considerably between species (19, 38). In addition, while mice are commonly used as models to study human diseases, such as hypertension, the exact role of PGI2 synthesis in their renal arteries still remains to be clearly elucidated. Moreover, there is considerable inconsistency regarding the role of each of the two COX isoforms in regulating vasomotor reactions, including those of renal vasculature (6, 8, 12, 17, 27, 29, 34, 35, 37, 38, 40), possibly in part due to the use of isoform-selective COX inhibitors that might have effects independent of their intended targets (2, 3, 20, 26).

Therefore, the aim of this study was to determine whether PGI2 evokes contraction in mouse renal arteries and, if so, how TP and/or IP receptors function in the vessel. In addition, experiments were performed to determine how the genetic deficiency of COX-1 (COX-1−/−) influences on the in vitro PGI2 synthesis and/or vasomotor reaction.

MATERIALS AND METHODS

Chemicals and solution. Nω-nitro-L-arginine methyl ester (L-NAME), ACh, phenylephrine (PE), AA, and the nonselective COX inhibitor indomethacin were purchased from Sigma (St. Louis, MO). PGI2, the stable PGI2 analog and IP receptor agonist iloprost, the stable PGI2 metabolite 6-keto-PGF1α, the IP receptor antagonist CAY10441, and the TP receptor antagonist SQ29548 were purchased from Cayman Chemical (Ann Arbor, MI). L-NAME, PE, ACh, 6-keto-PGF1α, and AA were dissolved in distilled water (purged with N2 for dissolving AA), while PGI2 or iloprost was dissolved in carbonate buffer (50 mM; pH 10.5; at 2,000-fold working concentration and used immediately after dissolving) and physiological salt solution (PSS), respectively. Indomethacin, CAY10441, and SQ29548 were
dissolved in DMSO at 2,000-fold working concentration [the final concentration of DMSO was 0.05% (vol/vol)].

The compositions of PSS and 60 mM K⁺-PSS (K⁺) were as described previously (21).

Mice and tissue preparation. All procedures performed on mice were in conformance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and approved by the Institutional Animal Research and Use Committee of Shantou University.

C57BL/6 mice were obtained from SLAC (Shanghai, China). Heterozygous COX-1-deficient (COX-1+/−; C57BL/6 background) breeders were kindly provided by Dr. Youfei Guan (Beijing University Medical Center). COX-1+/− mice were produced by cross-breeding male COX-1+/− with female COX-1+/− mice. Genotyping was performed by tail biopsy PCR as described elsewhere (18, 21). All mice were normal in appearance and without any overt cardiovascular abnormality.

Male C57BL/6 and/or COX-1+/− mice (16–20 wk) were euthanized by CO₂ inhalation. With the assistance of a binocular microscope, the main renal artery (the major stem) from either side was dissected free of adherent tissue. For the functional studies, the vessel was cut into 1-mm rings as described previously (21). In some experiments, carotid arteries that have limited IP receptor function and show prominent contraction to endothelial agonists, such as ACh and/or mesenteric arteries, which show potent relaxation to PGI₂, from C57BL/6 mice were prepared for control functional analyses (19, 21).

Analyses of vascular response. In vitro analyses of vascular function were performed with isometric force measurement as described elsewhere (21). Briefly, the vascular ring was mounted between two tungsten wires in an organ bath filled with PSS aerated with 95% O₂-5% CO₂ and maintained at 37°C. One wire was stationary, whereas the other was connected to a force transducer (AE 801). Thereafter, vessels were stimulated with 60 mM K⁺ every 15 min, and the resting tension was increased in a stepwise manner. After the equilibration, the resting tension was adjusted to an optimal level (−250 mg), at which the response to 60 mM K⁺ was maximal and reproducible.

In some experiments, vessels were treated with the NO synthase (NOS) inhibitor L-NAME (1 mM), under which the response of arteries appears similar to that in eNOS−/− mice (43). L-NAME, indomethacin, CAY10441, or SQ29548 was added 30 min before the vessel was contracted with an agent and was kept in solution throughout the experiment. For the control response of an inhibitor, the experiment was performed under the same conditions except that the inhibitor was replaced with the vehicle alone (water for L-NAME while DMSO for indomethacin, CAY10441, or SQ29548; 1.5 µL of vehicle each for 3 ml of PSS). The response elicited by an agonist under the baseline condition was expressed relative to that of 60 mM K⁺, while that during the contraction evoked by PE at the amount indicated was expressed relative to the value immediately before the application of the agent.

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Assay of 6-keto-PGF₁α. The production of the PGI₂ metabolite 6-keto-PGF₁α in renal arteries was detected with enzyme immunoassay (EIA) as described elsewhere (21). Renal arteries from both sides were pooled for each single experiment. With the help of a microscope, vessels were cut open and in some experiments, the endothelium was removed with a moistened cotton swab. Vessel strips were incubated with 150 µL PSS at 37°C for 30 min, followed by 150 µL PSS (basal) and that containing ACh (10 µM) 15 min each. Thereafter, reaction solutions were taken out and diluted 1:10 with PSS for 6-keto-PGF₁α detection. Measurements with EIA kits (Cayman Chemical, Ann Arbor, MI) were performed according to the manufacturer’s instructions (21). The amount of 6-keto-PGF₁α was expressed in nanograms per milligram of wet tissue.

Protein detection. Expressions of PGI₂ synthase (PGIS), COX-1, and GAPDH (as controls) were detected by Western blot. For protein preparation, vessel specimens (pooled from 4 mice for each single set of experiments) were cut open and rinsed of any blood components, followed by mincing and homogenizing in ice-cold RIPA buffer containing proteinase inhibitor cocktail (Roche Applied Science, Mannheim, Germany) with a glass homogenizer. Anti-PGIS (polyclonal; rabbit; 1:2,000 dilution) and anti-COX-1 (polyclonal; rabbit; 1:2,000 dilution) were used as first antibody (EIA) as described elsewhere (21).

Fig. 1. Vasomotor reaction to prostaglandin I₂ (PGI₂) in mouse renal arteries (RA). A: representative recordings (each from 3–5 replicates) showing the response evoked by 0.003–0.03 µM PGI₂ in intact RA precontracted with 1 µM phenylephrine (PE; top) or that evoked by 0.03–10 µM PGI₂ in Nω-nitro-L-arginine methyl ester (L-NAME)-treated RA under baseline conditions. B: Concentration-response curves of PGI₂ in L-NAME-treated RA showing the effect of thromboxane-prostanoid (TP) receptor antagonist SQ29538 (10 µM) in L-NAME-treated RA and compared with that of carotid arteries (CA). C and D: comparison of the concentration-response curves of the TP receptor agonist U46619 or PE between L-NAME-treated RA and CA. In B–D, values were expressed as means ± SE (n = 5 for each). ** P < 0.01.
Iloprost 0.1 µM

B

am sections. After placement on positively charged kidneys were immersed in 10% formalin, embedded in paraffin, and detected by immunohistochemistry. Briefly, mouse renal arteries or using a Kodak X-ray film (XBT-1; Xiamen, China).

ECL Plus kit (Amersham, Buckinghamshire, UK) and were detected Immunocomplexes were visualized with reaction solution from an manufacturer’s instructions. First-strand cDNA was synthesized using total glass homogenizer. Total RNA was prepared according to the manufac-

tor’s instructions. First-strand cDNA was synthesized using total RNA (250 ng) and oligo(dT)_{18} primers (TakaRa). The PCR primers for TP receptor, IP receptor, and α-actin were described previously (19). The specificity of primers was first confirmed with regular RT-PCR. Real-time PCR was performed using a SYBR PrimScript RT-PCR kit (TakaRa).

Data analysis. Data are expressed as means ± SEM from n numbers or pools of vessels from different animals. The Student’s t-test (unpaired; two tails) was used to compare the difference between two means for statistical evaluation. When more than two means were compared, one-way ANOVA followed by the Dunnett’s post hoc test or two-way ANOVA with Bonferroni’s post-hoc test was used. P < 0.05 was considered to be statistically significant.

RESULTS

Response to PGI2 in mouse renal arteries. The effect of PGI2 was first examined on vessels that had been contracted with PE. As shown in Fig. 1A, in intact or L-NAME-treated (data not shown) C57BL/6 mouse renal arteries contracted with PE (0.1 or 1 µM, respectively, to achieve 30–40% of the contraction evoked by 60 mM K+). PGI2 at 0.003 µM did not evoke relaxation but instead elicited a slight increase of force, which became evident when 0.03 µM of the agent were applied. Moreover, in L-NAME-treated vessels PGI2 (from 0.03 µM) evoked a concentration-dependent contraction.

Fig. 2. Response to PGI2 or iloprost after TP receptor antagonism. A: representative traces (left) and summary of results (right, n = 5 for each) showing the effect of PGI2 on L-NAME-treated RA or CA contracted with 2 µM PE in the presence of TP receptor antagonist SQ29548 (10 µM). B: representative traces (left) and summary of results (right; n = 5 for each) showing the abolition of the relaxation evoked by 1–10 µM PGI2 on L-NAME-treated CA, preconstricted with 2 µM PE in the presence of SQ29548 (SQ; 10 µM) by the IP receptor antagonist CAY10441 (+CAY, 0.3 µM). C: representative traces (left) and summary of results (right; n = 5 for each) showing the effect of iloprost (1 µM) on RA, CA, or mesenteric arteries (MA) treated in a similar manner as in A. Values were expressed as means ± SE. **P < 0.01.
which was inhibited by 10 μM of the TP receptor antagonist SQ29548 but was more potent than that in carotid arteries, under baseline conditions (Fig. 1B). It should be noted that in such conditions the only immediate PG12 metabolite 6-keto-PGF1α (1 μM) was unable to induce any contraction (data not shown), concurring with results reported on porcine interlobular renal arteries (22). In addition, the contraction evoked by the TP receptor agonist U46619 in such treated condition was also more potent in renal than in carotid arteries (Fig. 1C), while that evoked by PE was not significantly different between the two vessel types (Fig. 1D).

Response to PG12 or iloprost on PE-induced contraction after TP receptor antagonism. In vessels showing robust contraction to PG12, IP receptors may have, if any, only limited functional involvement (19, 21, 22). Therefore, we determined the effect of PG12 or its stable analog and the IP receptor agonist iloprost on mouse renal arteries after TP receptor blockade. Again, the carotid artery, which has been suggested to have only limited IP receptor-mediated activity that is masked by that of TP receptors, and/or the mesenteric artery, where IP receptors showed robust dilator activity, were used as controls (19). Vessels were precontracted with 2 μM PE (to achieve a sustained contraction with 90–110% of that evoked by 60 mM K+ in the presence of L-NAME and TP receptor antagonist SQ29548 (10 μM)).

As shown in Fig. 2A, PG12, which evoked minor relaxation in carotid arteries at concentrations of 0.1–10 μM, did not evoke any relaxation in renal arteries. It should be noted that in renal arteries that had been contracted with a lower concentration of PE (0.1 μM; ~30–40% of contraction evoked by 60 mM K+), PG12 did not evoke any significant relaxation as well (n = 3; data not shown). Also, the relaxation evoked by PG12 in carotid arteries was abolished by 0.3 μM of the IP receptor antagonist CAY10441 (Fig. 2B). Moreover, iloprost (0.1 μM), which evoked a minor relaxation in carotid and an almost complete relaxation in mesenteric arteries, did not cause any dilator effect on the renal arteries (Fig. 2C).

Expression of PGIS and/or PG12 synthesis in renal arteries. Next, we determined whether PG12 could be synthesized locally by the renal vasculature. As previously shown in some other vascular beds (21, 22), in mouse renal arteries the immunocomplex of PGIS could be clearly seen in endothelium as well as in medial smooth muscle (Fig. 3A). In addition, PGIS was detected in small intrarenal arteries (Fig. 3B). Moreover, Western blot showed that the expression level of PGIS was similar between the renal and carotid arteries (Fig. 3C).

COX-1 mediates endothelial PG12 synthesis in many other vascular beds (19, 22, 42). Thus the production of the PG12 metabolite 6-keto-PGF1α evoked by the endothelial muscarinic receptor agonist ACh in C57BL/6 or COX-1−/− renal arteries was examined. In C57BL/6 vessels, ACh (10 μM) evoked a ~10-fold increase in the production of 6-keto-PGF1α; however, the amount was reduced to near basal levels either in endothelium-denuded or in COX-1−/− vessels (Fig. 3D). In addition, the level of 6-keto-PGF1α in COX-1−/− vessels under basal conditions was also significantly lower than that of C57BL/6 mice (Fig. 3D).

Vasomotor reaction evoked by ACh in C57BL/6 and COX-1−/− renal arteries. At the same time, we determined vasomotor reactions evoked by ACh in C57BL/6 and COX-1−/− renal arteries. Under baseline conditions, ACh was not able to evoke any response with or without the presence of L-NAME (data not shown). Interestingly, in L-NAME-treated vessels contracted with PE (2 μM), ACh (10 μM) induced relaxation, which was blunted by a force increase (Fig. 4, A, top, and B). However, COX-1−/− (Fig. 4, A, middle, and B) or 10 μM of the TP receptor antagonist SQ29548 (Fig. 4C) impeded the force increase, resulting in enhanced relaxation evoked by ACh (Fig. 4, B and C). In addition, such relaxation in COX-1−/− vessels was unaltered by the nonselective COX
inhibitor indomethacin (Fig. 4B) but abolished in vessels contracted with 60 mM K+ (Fig. 4, A, bottom; n = 3).

**Response evoked by AA in C57BL/6 and COX-1−/− renal arteries.** To further confirm the functional predominance of COX-1 and determine whether non-COX AA metabolites, which have been suggested to be endothelium-derived hyperpolarizing factors (EDHF), cause relaxation in mouse renal arteries (10, 15, 41), the responses evoked by AA in vessels from C57BL/6 and COX-1−/− mice were analyzed. Vessels were also treated with l-NAME (1 mM) and contracted with PE (2 µM). In C57BL/6 vessels, 3 µM AA evoked an increase of force (Fig. 5, A, top, and B), which was absent in COX-1−/− mice (Fig. 5, A, middle, and B). Interestingly, in such treated COX-1−/− vessels AA did not evoke relaxation, which was in contrast to the response evoked by subsequent application of ACh (Fig. 5A, middle). In addition, the nonselective COX inhibitor indomethacin did not show any effect on COX-1−/− vessels (Fig. 5, A, bottom, and B).

**Expression of COX-1, TP or IP receptors in mouse renal arteries.** Lastly, the expressions of COX-1, TP and IP receptors in mouse renal arteries were examined. As positive controls, those of mouse carotid arteries were also examined. As shown in Fig. 6A, the expression of COX-1 was clearly detected by Western blot in mouse renal arteries, although the level was lower than that of the carotid arteries. In addition, both TP and IP receptor mRNAs were not only detected in mouse renal arteries but were also at higher levels than in carotid arteries (Fig. 6B).

**DISCUSSION**

In this study, we demonstrate for the first time that in mouse renal arteries PGI2 evokes contraction more potently than in carotid arteries. A similar result is observed with the TP receptor agonist U46619. However, in mouse renal arteries TP receptor antagonism, which inhibits the contraction, did not result in relaxation in response to PGI2. Moreover, we note that
the endothelial agonist ACh induces an increase in the production of the PGI2 metabolite 6-keto-PGF1α, which is abolished by endothelial denudation or by COX-1−/−. Interestingly, COX-1−/− also obliterates a force development that was sensitive to TP receptor antagonism and results in enhanced relaxation evoked by ACh following NOS inhibition. These results not only demonstrate a potent vasoconstrictor effect evoked by PGI2 but also suggest its synthesis via COX-1 plays an important role in the endothelium-derived vasoconstrictor activity of mouse renal arteries.

The ability of PGI2, which is able to activate both IP and TP receptors (19), to evoke a vasoconstrictor response is clearly demonstrated by an increase of force it evoked on PE-induced contraction in NOS-intact renal arteries. Of note, PGI2 also evoked contraction under baseline condition. In fact, the contraction to PGI2 in l-NAME-treated renal arteries is stronger than that in carotid arteries, where limited function of IP receptors is masked by the activity of TP receptors (19, 21). A similar differential contraction is obtained with the TP receptor agonist U46619; however, the α1-adrenergic receptor agonist PE evokes very similar contractions between the two vessel types. Also, real-time PCR showed that the mRNA level of TP receptors is higher in renal than in carotid arteries. These results indicate a selective high expression of TP receptor in mouse renal arteries. On the contrary, after TP receptor antagonism, which inhibits the contraction, PGI2 results in a minor relaxation in carotid but not in renal arteries. Again, a similar result is observed with iloprost, a stable PGI2 analog and IP receptor agonist that also activates TP receptors (22). It should be noted that following TP receptor antagonism iloprost evokes a strong relaxing effect on mesenteric arteries as PGI2 did previously (19). In addition, the relaxation to PGI2 in carotid arteries is inhibited by IP receptor antagonism. Also, our results with 6-keto-PGF1α rule out that the contraction to PGI2 was mediated through a degraded product. Therefore, in mouse renal arteries the observed effect with PGI2 reflects a high expression of TP receptors together with little functional involvement of the vasodilator IP receptors.

Also worthy of mention was that, in mouse renal arteries, PGIS is detected in a similar manner as in some other mouse arteries (21). Indeed, the endothelial muscarinic receptor agonist ACh evoked an increase in the production of the PGI2 metabolite 6-keto-PGF1α, which is abolished by endothelial denudation or by COX-1−/−. Moreover, COX-1−/− is further noted to obliterate force development that is sensitive to TP receptor antagonism and result in enhanced relaxation evoked by ACh following NOS inhibition. Also, this effect of COX-1−/− was not enhanced by the addition of nonselective COX inhibition. Thus COX-1, which has been commonly found to be expressed in endothelium (11, 21, 22), appears as a major functional COX isofrom, which mediates both endothelium-dependent PGI2 synthesis and vasoconstrictor activity evoked by ACh. It should be noted that the amount of PGI2 evoked by ACh was ~1.9 ng/mg, well above the level (3 nM) to initiate vasoconstrictor activity (2 ng/mg of 6-keto-PGF1α can be translated into 5.4 μmol PGI2 per kg of wet vessel). In addition, the intermediate COX product PGH2, which could be converted to PGI2 in medial smooth muscle where PGIS was also identified in the present study, acts on TP receptors (11, 13, 42). As a result, PGI2 synthesis itself may lead to TP receptor activation in mouse renal arteries and hence play an important part in the vasoconstrictor activity evoked by ACh. In agreeing with this point, the COX substrate AA, which stimulates endothelium-dependent PGI2 synthesis (19, 42), also evokes contraction in a manner that is abolished in COX-1−/− renal arteries.

We note that even in L-NAME-treated mouse renal arteries ACh did not cause contraction under baseline conditions. This could be due to a masking effect of the concomitantly activated NO-independent dilator activity on COX-1-mediated vasoconstrictor reaction. Interestingly, in carotid arteries where the NO-independent dilator response is also activated, ACh still induces a prominent contraction (21, 42). Part of the reason for this could be the altered level of COX-1, which is found to be lower in renal than in carotid arteries. Moreover, we find that the relaxation evoked by ACh in l-NAME-treated COX-1−/− vessels is abolished by 60 mM K+, concurring with the dilation mediated by EDHF previously shown in mouse vascular beds (1, 10, 24). On the other hand, our results with AA in COX-1−/− vessels may not only imply little function for COX-2 but also suggest that non-COX AA metabolites, which are proposed to function as EDHF in mouse vascular beds and vessels of some other species (10, 15, 41), have little role in mouse renal arteries under the in vitro experimental condition, consistent with findings we previously reported in several other mouse vessels (42). It should be noted that although non-COX
AA metabolites have been considered to act as EDHF in renal vasculature (15), a direct vasodilator effect of AA on isolated renal arteries following COX blockade has not yet been reported, possibly resulting from conditions that are different from those of in vivo experiments.

Therefore, our results with vessels from COX-1−/− mice further demonstrate that the process of native PGI2 synthesis leads to vasoconstrictor activity and that COX-1, although it appears lower than that in some other mouse vascular beds, is the major AA-metabolizing enzyme in mouse renal arteries. These findings concur with our previously obtained results in porcine interlobular arteries (22). Indeed, intra-aortically applied PGI2 has been found to reduce the flow rate of rat superficial nephrons, although this was explained by increased activity of the renin-angiotensin system (31). Moreover, our results further revealed that in carotid arteries, where the limited function of IP receptor is masked by activity of TP receptors, PGI2 evoked a minor relaxation, only at concentrations of 1–10 μM, following TP receptor antagonism. At the same time, in renal arteries the lowest concentration for PGI2 to evoke contraction was ∼3 nM. Interestingly, we recently showed that in mouse mesenteric arteries where IP receptors mediate potent dilator activity, the initial concentration of PGI2 showed that in mouse mesenteric arteries where IP receptors; hence, like that of IP receptors, TP receptor activation could be an integral part of PGI2 synthesis associated with endothelium-mediated regulation of local vascular function.

On the other hand, although PGI2, a commonly recognized vasodilator, is found to mediate contraction in a variety of vascular beds (11, 21, 22, 36, 38), its constrictor effect on renal arteries could be species specific or could even vary with the site of a vessel segment (22, 37, 39). In addition, the level of IP receptor mRNAs is higher in renal than in carotid arteries, contradicting the results from functional analyses. Notably, PGI2 has also been reported not to evoke dilator activity in hypertensive rat aortas with an unaltered IP receptor expression (39). Thus there might be an undefined mechanism(s) refraining IP receptors from mediating a dilator activity. However, the amount of mRNAs may not reflect the receptors at protein level. Unfortunately, we were unable to detect IP receptors with Western blot in mouse arteries, due to limited sensitivity of the method. As a result, the reason(s) for the inconsistency between mRNA level and functional involvement of vasodilator IP receptors in PGI2-mediated response remain to be elucidated. Meanwhile, although the vasoconstrictor effect PGI2 synthesis might be insignificant compared with the vasodilator activities of the endothelium, its impact can significantly increase in diseases, such as hypertension (7, 11, 36, 38). In addition, our result suggest that PGI2 could also be produced in intrarenal arteries, although a dilator activity might predominate over the constrictor response to PGI2 or COX-mediated metabolism in some intra-renal arteries (39).

In summary, in the present study with in vitro functional and biochemical analyses, our results demonstrate that in mouse renal arteries high expression of TP receptors together with little functional involvement of the vasodilator IP receptors results in a potent vasoconstrictor effect evoked by PGI2. In addition, our data suggest that the process of COX-1-mediated native PGI2 synthesis can also lead to vasoconstrictor activity and this could be an integral part of endothelium-derived mechanisms that regulate local renal vascular function.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: B.L. and Y. Zhou conception and design of research; B.L., Y. Zhang, N.Z., and H.L. performed experiments; B.L., Y. Zhang, N.Z., H.L., W.L., and Y. Zhou analyzed data; B.L., Y. Zhang, W.L., and Y. Zhou interpreted results of experiments; B.L., Y. Zhang, W.L., and Y. Zhou prepared figures; B.L. and Y. Zhou drafted manuscript; B.L. and Y. Zhou edited and revised manuscript; B.L., Y. Zhang, N.Z., H.L., W.L., and Y. Zhou approved final version of manuscript.

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