Role of cyclooxygenase-1-mediated prostacyclin synthesis in endothelium-dependent vasoconstrictor activity of porcine interlobular renal arteries

Bin Liu,1 Wenhong Luo,2 Yingzhan Zhang,1 Hui Li,2 Ningxia Zhu,1 Dongyang Huang,3 and Yingbi Zhou1
1Cardiovascular Research Center, 2The Central Laboratory, and 3Department of Molecular and Cellular Biology, Shantou University College of Medicine, Shantou, China

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Liu B, Luo W, Zhang Y, Li H, Zhu N, Huang D, Zhou Y. Role of cyclooxygenase-1-mediated prostacyclin synthesis in endothelium-dependent vasoconstrictor activity of porcine interlobular renal arteries. Am J Physiol Renal Physiol 302: F1133–F1140, 2012. This study aimed to determine whether PGI2 would be evoked by the endogenous endothelial B2 receptor agonist bradykinin (BK) in the porcine interlobular renal artery and, if so, to determine how it would influence the vasomotor reaction, and the specific cyclooxygenase (COX) isomform(s) involved in its synthesis. The production of the PGI2 metabolite 6-keto-PGF1α was analyzed with HPLC-mass spectroscopy, while vasomotor reaction to PGI2 or BK was determined with isometric force measurement. Results showed that BK evoked an increase in the production of 6-keto-PGF1α, which was abolished by endothelial denudation that removed COX-1 expression, or was reduced by COX-1 inhibition. Interestingly, PGI2 evoked a potent contraction, which was prevented by antagonizing thromboxane-prostanoid (TP) receptors and was not enhanced by antagonizing the vasodilator PGH2 IP receptors. The IP receptor agonists MRE-269 and iloprost did not induce any relaxation. Moreover, iloprost, which is also a PGI2 analog, caused a contraction, which was sensitive to TP receptor antagonism, but was to a significantly lesser extent than that of PGI2. Indeed, IP receptors were not detected by RT-PCR or Western blotting in the vessel. Following nitric oxide synthase (NOS) inhibition, BK also evoked an endothelium-dependent contraction, which was blocked by TP receptor antagonism. In addition, inhibition of COX-1 (but not COX-2) impeded the vasoconstrictor activity of BK and expedited the relaxation induced by the agonist in NOS-intact vessels. These results demonstrate that in the porcine interlobular renal artery BK evokes endothelial COX-1-mediated PGI2 synthesis, which mainly leads to the activation of TP receptors and a vasoconstrictor response, possibly due to a scarcity of vasodilator activity mediated by IP receptors. Also, our data suggested that the effect of a PGI2 analog on TP receptors could be reduced compared with that of PGI2 due to modified structure as with iloprost.

COX isoform; TP receptor; renal vasculature; contraction

Cyclooxygenases (COX) metabolize arachidonic acid to produce vasoactive prostanooids. Among them, thromboxane (TX) A2 acts on TX-prostanoid (TP) receptors to mediate vasoconstriction, whereas prostacyclin (PGI2) on PGI2 (IP) receptors mediates vasodilation and protects vessels from the development of diseases (6, 34). TX-A2 and PGI2 have been considered to be produced in platelets and vascular endothelium, respectively (4, 6, 25, 34). In biological tissues, COX enzymes exist mainly in COX-1 and COX-2 isoforms. While COX-2 has been proposed to be the major mediator of endothelial PGI2 synthesis (9, 11), there are studies indicating COX-1 as playing a predominant role in some vascular beds (3, 26, 35). Also, in some vascular beds the production of PGI2 is linked to TP receptor activation and is endothelium dependent (8, 10, 21–23, 41, 42); however, such an effect of PGI2 has been mainly reported in large arteries or in rat and mouse vessels that are structurally or functionally corresponding to large or conduct arteries in humans.

Intrarenal blood flow plays an important role in controlling systemic blood pressure and balance of body fluid and salts. In the renal vasculature, PGI2 is proposed to be a major endothelium-derived COX metabolite that mainly functions to mediate vasodilation; decreased production of PGI2 has been suggested to be implicated in endothelial dysfunction under disease condition (11, 16, 30). However, direct analysis of endothelial PGI2 synthesis by intrarenal arteries has not yet been performed. Notably, in the porcine interlobular renal artery the endothelial B2 receptor agonist bradykinin (BK) has been reported to evoke endothelium-dependent vasoconstrictor activity that was sensitive to COX inhibition or TP receptor antagonism (13, 14). Therefore, there is a pressing need to determine whether the production of PGI2 would be evoked by BK and, if so, how it would affect the vasomotor reactivity of intrarenal arteries. Also, both COX-1 and COX-2 have been suggested to mediate prostanoid synthesis in kidneys (12, 18); however, the role of each in endothelial PGI2 synthesis of the renal vasculature has not yet been clearly established.

To address the above issues, porcine interlobular renal arteries, which have sizes similar to those in humans, were isolated for biochemical and functional analyses. The PGI2 metabolite 6-keto-PGF1α was analyzed with HPLC-mass spectroscopy (MS), while the vasomotor reaction to PGI2 or BK was determined with isometric force measurement.

Materials and methods

Chemicals and solutions. Nω-nitro-l-arginine methyl ester (l-NAME), phenylephrine (PE), BK, and the selective COX-2 inhibitor celecoxib were purchased from Sigma (St. Louis, MO). Selective COX-1 inhibitors FR122047 and SC560 were purchased from Calbiochem (La Jolla, CA). PGI2, 6-keto-PGF1α, the IP receptor agonists iloprost and MRE-269, antagonist CAY10441, and the TP receptor antagonist SQ29548 were bought from Cayman Chemical (Ann Arbor, MI). l-NAME, BK, PE, 6-keto-PGF1α, and FR122047 were dissolved in distilled water, while PGI2 or iloprost was dissolved in carbonate buffer (50 mM; pH 10.5) and physiological salt solution (PSS), respectively. Celecoxib, SC560, CAY10441, MRE-269, and SQ29548 were dissolved in DMSO at 2,000-fold of the final working concentration. To selectively and completely inhibit the activity of COX-1, 1 μM FR122047 (IC50 values of 0.028 and 65 μM for COX-1 and COX-2, respectively) or 0.3 μM SC560 (IC50 values of 0.009 and 6.3 μM for COX-1 and COX-2 respectively) was used (27, 33). Also, the concentration of celecoxib (3 μM), CAY10441 (1 μM), iloprost,

Address for reprint requests and other correspondence: Y. Zhou, Cardiovascular Research Center, Shantou Univ. College of Medicine, 22 Xin-Ling Rd., Shantou, China 515041 (e-mail: zhouchenyihji35@gmail.com).
MRE-269 (0.1–1 μM), or SQ29548 (10 μM) was based on previous reports, in which those compounds were suggested to effectively act on their respective intended enzymes or receptors (8, 19, 20, 23, 28, 40, 44).

The compositions of PSS and 60 mM K⁺-PSS (K⁺) were as described previously (44, 45).

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

Pig kidneys were purchased from local slaughterhouse and transported to the laboratory in ice-cold PSS. Kidneys were cut open, and the interlobar renal arteries with an internal diameter of <1 mm were isolated for experimental purposes. With the assistance of a binocular microscope, vessels were dissected free of adherent tissues. For functional analysis, vessels were further cut into 1-mm rings. To detect protein and mRNA expressions or analyze COX metabolites in endothelium-denuded specimens, vessels were cut open, and the endothelium was removed with a razor blade.

**Assay of 6-keto-PGF₁α.** The PGI₂ metabolite 6-keto-PGF₁α was directly analyzed with HPLC-MS, with which signals from molecular (23). Briefly, porcine interlobar renal arteries were incubated with endothelium-denuded specimens, vessels were cut open, and the endothelium was removed with a razor blade. The same extraction procedure, and the amounts of 6-keto-PGF₁α was maximal and reproducible. In control experiments, inhibitors were replaced with the solvent. In accordance with previous studies, the response to an agonist under the baseline condition was expressed relative to the contraction evoked by 60 mM K⁺, while that during PE-induced contraction was expressed as a change in force relative to the value immediately before the agent was applied (7, 14, 15, 23).

**Protein detection.** The expressions of PGI₂ synthase (PGIS), IP receptors, or β-actin (as an internal control) were detected by Western blotting. Anti-PGIS (polyclonal; rabbit; 1:2,000) and anti-IP receptor (polyclonal; rabbit; 1:2,000) antibodies were purchased from Cayman Chemical, while anti-β-actin antibody (polyclonal; rabbit; 1:2,000) was bought from Santa Cruz Biotechnology (Santa Cruz, CA). Immunocomplexes were visualized with reaction solution from an ECL plus kit (Amersham, Buckinghamshire, UK), and detected using Kodak X-ray film (XBT-1; Xiamen, China). The probing of β-actin was performed on membranes where IP receptors had been detected, with the original antibodies being stripped off using a buffer containing 62.5 mM Tris·HCl (pH 6.8), 2% SDS, and 0.8% β-mercaptoethanol (17). The molecular weights of IP receptor, β-actin, and PGIS were ~52,000, 43,000, and 57,000, respectively (2, 23).

PGIS, which appeared as a single band in Western blotting, was also detected with immunohistochemistry. Briefly, porcine interlobar renal arteries were immersed in 10% formalin, embedded in paraffin, and sliced into 5-μm sections. After placement on positively charged slides, sections were treated with 3% hydrogen peroxide for 20 min following by blocking with 1.5% normal goat serum. Subsequently, the slides were incubated with anti-PGIS antibody (1:500 dilution; polyclonal, Cayman Chemical) and then exposed to a biotinylated secondary antibody. For the negative controls, sections were treated in a similar manner except that the anti-PGIS antibody was omitted from the reaction. The antibody-antigen complexes were identified through incubation with diaminobenzidine, and sections were counterstained with hematoxylin.

**RT-PCR.** The expressions of IP, TP receptors, COX-1, and β-actin (as an internal control) were performed with RT-PCR. RNA preparation was performed using an RNAiso Plus (TaKaRa, Dalian, China) kit, according to the manufacturer’s instruction. Moloney marine leukemia virus reverse transcriptase and Oligo(dT)₁₄ primer (Promega, Madison, WI) were used to synthesize first-strand cDNAs. The RT reaction was performed with 200 ng of total RNA in a volume of 20 μl. PCR Primers were designed based on porcine (IP receptor, COX-1, and β-actin) or bovine (TP receptor) cDNA sequences: 5′-GGA CCT CCT CGC CCT CGG TTT C-3′ (sense) and 5′-TGG CGT TCC CAC GGT GTT GCC 3′- (antisense) for IP receptors; 5′-CGG CTC CCT CGG TGT CCT CTT C-3′ (sense) and 5′-TGC AGG ATC TGG TCC GAT GTG 3′- (antisense) for TP receptors; 5′-ACA GTG GTT TCC AAC CTT ATC ACC 3′- (sense) and 5′-TGA GTT TCC CAT CCT TAA AGA GCC 3′- (antisense) for COX-1; and 5′-TGG GGC ATC CAT GAA ACT AC 3′- (sense) and 5′-GAA TGC AAC TAA CAG TGG GCC TGC 3′- (antisense) for β-actin. The thermal cycle protocols for PCR were as follows: 94°C for 30 s, 55°C for 30 s, 72°C for 30 s (28 cycles for β-actin; 32 cycles for IP and TP receptors; 36 cycles for COX-1). The expected sizes of PCR products were 301 bp for IP receptors, 375 bp for TP receptors, 406 bp for COX-1, and 335 bp for β-actin. PCR products were separated on 2% agarose gels and visualized with ethidium bromide staining.

**Data analysis.** Data were expressed as means ± SE in vessels from n numbers of animals. Student’s t-test (unpaired; 2 tailed) was used to compare the difference between two means. When more than two means were compared, one-way or two-way ANOVA followed by Dunnett’s or Bonferroni’s post hoc test, respectively, was used. P < 0.05 was considered to be statistically significant.

**RESULTS**

**Production of PGI₂ in porcine interlobar renal artery.** In HPLC-MS, MW370s (6-keto-PGF₁α and TxB₂), MW354 (PGF₂α), or...
MW352s (PGE₂ and PGD₂) could be simultaneously monitored, and a similar intensity of signals was observed with 10 ng PGF₂α, 25 ng 6-keto-PGF₁α, 50 ng PGE₂, or 100 ng TxB₂ (Fig. 1A, top; Fig. 1A, bottom), is a blank control in which only solvent peaks appear. In the sample solution, 6-keto-PGF₁α was the only COX-derived metabolite discernable (Fig. 1B, top), and its amount increased by about fivefold in vessels stimulated with 1 μM BK compared with that of basal condition (5.25 ± 0.45 vs. 1.1 ± 0.35 ng/mg; n = 5; P < 0.01). Endothelial denudation abolished basal 6-keto-PGF₁α production (data not shown) as well as that evoked by 1 μM BK (n = 3; Fig. 1B, bottom). In addition, the selective COX-1 inhibitor FR122047 (1 μM) not only abolished basal 6-keto-PGF₁α production but also reduced that evoked by BK (1 μM; Fig. 1, C and D). In contrast, the selective COX-2 inhibitor celecoxib (3 μM) did not have a significant effect.

Effect of PGI₂ on the porcine interlobular renal artery. Since 6-keto-PGF₁α was abundantly produced in response to BK, we then determined whether PGI₂ would evoke vasoconstrictor activity. Experiments were first performed in vessels treated with L-NAME. As shown in Fig. 2, starting from 0.3 μM PGI₂ induced a concentration-dependent contraction with the maximum (at 30 μM) reaching 199.3 ± 3.7% of that evoked by 60 mM KCl. Interestingly, the TP receptor antagonist SQ29548 (10 μM) abolished the contraction (Fig. 2, A and B), whereas the IP receptor antagonist CAY10441 (1 μM) did not alter the contraction (Fig. 2A). Also, the PGI₂ metabolite 6-keto-PGF₁α (up to 10 μM) was found not to evoke any contraction (data not shown).

Moreover, PGI₂ even evoked a contraction when L-NAME was omitted from the solution. However, compared with that obtained with L-NAME, the maximal contraction was significantly decreased (117.0 ± 8.8%; n = 5), and no response was observed below 1 μM (Fig. 2C). Under such a condition, CAY10441 (1 μM) did not enhance the contraction but rather reduced that evoked by 30 μM PGI₂ (Fig. 2C).

Expression and function of IP receptors. To determine the underlying mechanism for the inability of IP receptor antagonist to enhance PGI₂-induced contractions, the expression and functional presence of IP receptors in the porcine interlobular renal artery were examined. RT-PCR and Western blotting showed that mRNAs (Fig. 3A, top) and proteins (Fig. 3A, bottom) of IP receptors were not detected in the vessels, while both were clearly seen in the renal medulla. On the other hand, in the vessel samples mRNAs or proteins of β-actin were similarly or more abundantly detected compared with those in the renal medulla. Moreover, our functional analyses revealed that the IP receptor agonist MRE-269 (0.1–1 μM) and iloprost (0.1–1 μM) was unable to induce relaxation of the contraction evoked by 0.5 μM PE in vessels treated with 1 mM L-NAME (60–90% of 60 mM K⁺-induced contraction; Fig. 3B). In addition, iloprost, which is also a PGI₂ analog, evoked a contraction that was sensitive to the TP receptor antagonist SQ29548 (10 μM); however, the response (with a maximum of 26.7 ± 3.8% at 30 μM) was to a significantly lesser extent than that evoked by PGI₂ (Fig. 3C).

Effect of BK on L-NAME-treated vessels. Similar to the ACh-induced response in mouse aortas, in the porcine interlobular renal artery BK has been demonstrated to cause a COX-dependent force increase, which was eventually subdued by a concomitant NO release (14, 23). Therefore, we undertook to determine whether BK could evoke contraction in L-NAME-treated vessels under the baseline condition as ACh in rat or mouse vessels (10, 23, 45). As shown in Fig. 4, from 0.01 μM, BK induced a
concentration-dependent contraction, with a maximal response reaching 79.6 ± 5.8% of that caused by 60 mM K⁺. However, in endothelium-denuded vessels, BK (0.01–10 μM) was unable to induce a contraction (Fig. 4A). Also, the contraction evoked by BK showed a biphasic or unsustained property and was inhibited by the TP receptor antagonist SQ29548 (10 μM; Fig. 4, B and C; results from the contractions evoked by 1 μM BK).

**Effect of COX-1 inhibition on BK-induced vasomotor reaction.** To determine whether a reduction in 6-keto-PGF₁α production would be associated with altered contraction to BK following NOS inhibition, we examined the effect of selective COX-1 inhibition on vessels treated with L-NAME. As shown in Fig. 5A, the selective COX-1 inhibitor FR122047 (1 μM) or SC560 (0.3 μM) completely abolished the contraction evoked by 1 μM BK. In contrast, the contraction to BK (1 μM) in the presence of the selective COX-2 inhibitor celecoxib (3 μM) was not significantly different from that in controls (P > 0.05) (Fig. 5A).

Since 6-keto-PGF₁α was measured in the absence of L-NAME, the effect of COX-1 inhibition on NOS-intact vessels was also examined. As in the prior study (14), in L-NAME-untreated vessels contracted with PE (3 μM; 90–120% of the contraction evoked by 60 mM K⁺), BK (0.1 μM) evoked a

![Diagram of concentration-dependent contraction](image)

**Fig. 2.** Effect of PGI₂ on porcine interlobular renal arteries. A: concentration-responses of PGI₂ obtained in the presence of Nω-nitro-L-arginine methyl ester (L-NAME). The controls (CTL), those with vasodilator PGI₂ (IP) receptor antagonist CAY10441 (1 μM; +CAY10441), or thromboxane-prostanoid (TP) receptor antagonist SQ29548 (10 μM; +SQ29548) are represented by ○, ○, and ●, respectively. B: representative recordings of the control contraction to PGI₂ (0.3–10 μM) in the presence of L-NAME (1 mM) and that with SQ29548 (+SQ29548, bottom trace). C: contraction to PGI₂ in the absence of L-NAME. The controls (CTL) and those with CAY10441 (1 μM; +CAY10441) are represented by ○ and ○, respectively. In A and C, values are means ± SE (n = 5 for each). **, *: P < 0.01 and P < 0.05, respectively, compared with ○ values.

**Fig. 3.** Expression and function of IP receptors. A: RT-PCR (top) and Western blot (bottom) detection of mRNAs or proteins of IP receptors (IP-R) and β-actin (as internal controls) in the porcine interlobular renal artery (RA) and renal medulla (RM; as positive controls). Each was a representative from at least 3 replicates. M: molecular weight marker. Expected sizes of PCR products were 301 bp for IP receptors and 335 bp for β-actin. B: representative recordings (each was at least from 3 replicates) showing the effect of iloprost (top) and MRE-269 (middle) on vessels contracted with PE. Bottom: control with DMSO. C: effect of iloprost under the baseline condition. ○ and ●, Control response and that obtained with the presence of TP receptor antagonist SQ29548 (10 μM), respectively. In B and C, vessels were treated with L-NAME (1 mM). Values are means ± SE (n = 5 for each). *P < 0.05, **P < 0.01.
relaxation, which was blunted by a biphasic force increase with a peak force of 9.9 \pm 2.8\% above PE-induced contraction (Fig. 5B). Interestingly, FR122047 (1 \mu M) inhibited the force increase, resulting in an expedited BK-induced relaxation, which had time to reach half of the maximal relaxation (TR1/2) being significantly less than that of controls (Fig. 5B) \((P < 0.01)\).

Expression of PGIS, TP receptor, or COX-1. Finally, the expression of PGIS, TP receptors, or COX-1 in the porcine interlobular renal artery was determined. In Western blotting, PGIS was clearly seen in both the intact and endothelium-denuded arteries (Fig. 6A, left). Also, immunohistochemical staining showed that PGIS was present in the endothelium as well as in the media (Fig. 6A, right). In a similar manner, RT-PCR revealed that mRNAs of TP receptors were similarly detected in the intact and endothelium-denuded vessels (Fig. 6B, top). On the other hand, while mRNAs of COX-1 were clearly seen in the intact vessels, they were not detected in arteries that had been denuded of endothelium (Fig. 6B, bottom).

DISCUSSION

In this study, by using HPLC-MS we demonstrated the first direct evidence that in the porcine interlobular renal artery BK, which was associated with endothelium-dependent vasoconstrictor activity, evoked an increase in the production of PG\(_2\) metabolite 6-keto-PGF\(_{1\alpha}\) that was abolished or reduced by endothelial denudation or COX-1 inhibition, respectively. Interestingly, PG\(_2\) evoked a potent contraction, which was prevented by TP receptor antagonism that similarly inhibited the contraction evoked by BK following NOS inhibition. Moreover, inhibition of COX-1 impeded the vasoconstrictor activity evoked by BK and resulted in an expedited relaxation to the agonist in NOS-intact vessels. These results might suggest an intimate link between endothelial COX-1-mediated PG\(_2\) synthesis and TP receptor-mediated, endothelium-dependent vasoconstrictor activity in the porcine interlobular renal artery.
The presence of endothelium-dependent production of PGI₂ was clearly demonstrated by our HPLC-MS detection of the metabolite 6-keto-PGF₁α. In the porcine interlobular renal artery, 6-keto-PGF₁α was significantly increased following BK stimulation. This may suggest an essential involvement of PGI₂ synthesis in the regulatory mechanisms following the endothelial B₂ receptor activation. In addition, we found that inhibition of COX-1 reduced 6-keto-PGF₁α production, while that of COX-2 did not appear to have any effect. Also, similar to 6-keto-PGF₁α production, the expression of COX-1 was removed by endothelial denudation. These results indicate that the production of PGI₂ evoked by BK is in fact derived from COX-1-mediated metabolism in the endothelium. Moreover, the presence of PGIS detected by immunohistochemical staining implies that the process of PGI₂ synthesis could be accomplished inside the endothelium; however, as PGIS was also abundantly found in the media, there is a possibility that part of the endothelial COX-1-derived PGI₂ could be finally synthesized outside the endothelium.

Meanwhile, our functional analyses showed that PGI₂ evoked potent contraction of the porcine interlobular renal artery. Interestingly, the IP receptor antagonist CAY10441, which effectively inhibited the dilation to PGI₂ in rat aortas (8), did not enhance the contraction. Also, the IP receptor agonist MRE-269 or iloprost did not induce any relaxation. Moreover, RT-PCR and Western blotting showed IP receptors were not detected in the vessel. These results point to a scarcity of IP receptors in the vessel. On the contrary, the TP receptor antagonist SQ29548 (10 μM) abolished the contraction evoked by PGI₂, similar to results previously reported in some other vascular beds (8, 10, 21–23, 41, 42). Also, 6-keto-PGF₁α, the only immediate metabolite of PGI₂, was found not to induce any contraction, which excluded the possibility that the contraction evoked by PGI₂ was mediated through a degraded product. Therefore, in the porcine interlobular renal artery PGI₂ appears to act predominantly on TP receptors to mediate a potent vasoconstrictor effect. This, possibly resulting from the lack of a significant functional involvement by the vasodilator IP receptors (23, 38). Indeed, the TP receptors were similarly detected in vessels with or without endothelium, which was well in agreement with a major action of PGI₂ on the media smooth muscle as proposed elsewhere (10, 38). Also, the reduced contraction to PGI₂ in L-NAME-untreated vessels was possibly due to basal NO production, as observed in other studies (10). In addition, the inhibition of the IP receptor antagonist CAY10441 on the contraction evoked by 30 μM PGI₂ in L-NAME-untreated vessels could be a reflection of an unintended effect of the compound, resulting from a lenient selectivity of TP receptors, as demonstrated by results from prior studies (8, 10).

Another point of interest was that following NOS inhibition BK evoked an endothelium-dependent contraction that was sensitive to TP receptor antagonism. Again, such a BK-evoked response was abolished by COX-1 inhibition, but not by that of COX-2. Moreover, in NOS-intact vessels COX-1 inhibition impeded the biphasic force increase evoked by BK and resulted in an expedited relaxation. These results suggest that BK-evoked vasoconstrictor activity is similar to PGI₂ synthesis in the reliance on COX-1-mediated metabolism, and its existence is not a result of NOS inhibition. Also, the amount of 6-keto-PGF₁α evoked by BK (1 μM) could be translated into 4.7 μmol PGI₂ (molecular weight 352) per kg of wet vessels (3 times weight of dry tissues), which was above the concentration of PGI₂ to initiate vasoconstrictor activity. Moreover, PGI₂, an intermediate or precursor of PGI₂ that acts on TP receptors, has also been suggested to be implicated in the vessel (14). Therefore, in the vessel studied endothelial COX-1-mediated PGI₂ synthesis would mainly lead to the activation of TP receptors and hence could be largely responsible for the vasoconstrictor activity evoked by BK. On the other hand, we could not exclude a potential involvement of TXA₂, which could also be produced by the endothelium, although it might be of a minimal amount (10, 23, 40, 41) and was not detected in the present study. Meanwhile, the biphasic property of the contraction to BK could be explained by a self-limiting nature of COX activation and/or a rapid conversion of PGI₂ (t₁/2: 3 min) to a biologically inactive metabolite as put out previously (5, 25, 31, 32, 34).

Therefore, our above-noted results suggested an important role for COX-1-mediated PGI₂ synthesis in endothelium-de-
dependent contraction of the porcine interlobular renal artery. Traditionally, PGI2 has been considered to mediate dilation of intrarenal arteries (11, 16, 30). However, the present study was the first report to document the in situ PGI2 synthesis evoked by an endogenous agonist, BK, and relate it to endothelial COX-1-mediated metabolism in an intrarenal vessel. Moreover, our results suggest that PGI2 acts mainly on TP receptors to mediate a potent vasoconstrictor effect, possibly due to a scarcity of IP receptor-mediated vasodilator activity. A vasoconstrictor effect derived from COX-1-mediated PGI2 synthesis has previously been reported in rat or mouse arteries (1, 10, 23, 36, 37). However, those vessels were mostly corresponding to large or conduct vessels in humans. More importantly, the vasoconstrictor effect of PGI2 on the porcine interlobular renal artery was more potent than that previously reported in mouse or rat vessels (10, 23). This might underscore the potential impact of the above-mentioned unclassic effect of endothelial PGI2 synthesis on the renal vasculature. In addition, we noted that iloprost (an IP receptor agonist and PGI2 analog) caused a contraction that was to a significantly lesser extent than that of PGI2. This implies that the unfavorable effect of PGI2 on TP receptors could be reduced by modifying its structure as with iloprost, which is clinically used for pulmonary hypertension and peripheral arterial diseases (24, 29).

However, it needs to be pointed out that COX-1 has been suggested to mediate the dilation of mouse cerebral arteries or gracilis muscle arterioles (26, 35). Also, while COX-1 is commonly found to mediate endothelium-dependent contractions, there are also studies suggesting the involvement of only COX-2 or both COX-1 and COX-2 (10, 40–42). These findings might imply a diversified expression of endothelial COX isoforms and varied roles for the endothelial PGI2 synthesis in regulating local vascular function. Notably, in the rabbit renal afferent artery PGI2 has been found to be primarily responsible for the dilator response of BK (43), suggesting a segment-specific expression of IP receptors in the renal vasculature. On the other hand, in this vessel, BK also evoked COX-dependent vasoconstrictor activity (43); however, the exact underlying mechanism has not been elaborated on in the prior study. In addition, COX-1 or PGI2 synthesis has been suggested to account for endothelial dysfunction in ANG II-infused mouse (39) or hypertensive rat arteries (8, 38, 41, 42), respectively. Thus further studies are required to determine how endothelial PGI2 synthesis would affect the intrarenal vascular function under disease condition.

In summary, in this study by using HPLC-MS and isometric force measurement, we demonstrated that in the porcine interlobular renal artery BK evoked a COX-1-mediated increase in endothelium-dependent PGI2 synthesis, which on the other hand mainly leads to the activation of TP receptors and a vasoconstrictor response, possibly due to the paucity of IP receptor-mediated vasodilator activity. In addition, our data suggested that the effect of a PGI2 analog on TP receptors could be reduced compared with that of PGI2 due to modified structure as that of iloprost.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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

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