Prostaglandin I$_2$ and Prostaglandin E$_2$ Modulate Human Intrarenal Artery Contractility Through Prostaglandin E2-EP4, Prostacyclin-IP, and Thromboxane A2-TP Receptors

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Abstract— Cyclooxygenase inhibitors decrease renal blood flow in settings with decreased effective circulating volume. The present study examined the hypothesis that prostaglandins, prostaglandin E$_2$ (PGE$_2$) and prostacyclin (PGI$_2$), induce relaxation of human intrarenal arteries through PGE$_2$-EP and PGI$_2$-IP receptors. Intrarenal arteries were microdissected from human nephrectomy samples (n=53, median diameter ≈362 μm, 88% viable, 76% relaxed in response to acetylcholine). Rings were suspended in myographs to record force development. In vessels with K$^+$-induced tension (EC$_{50}$: –log [mol/L]=1.36±0.03), PGE$_2$ and PGI$_2$ induced concentration-dependent relaxation (–log EC$_{50}$ [mol/L]=7.1±0.3 and PGI$_2$=7.7). The response to PGE$_2$ displayed endothelium dependence and desensitization. Relaxation by PGE$_2$ was mimicked by an EP4 receptor agonist (CAY10598, EC$_{50}$=6.7±0.2). The relaxation after PGI$_2$ was abolished by an IP receptor antagonist (BR5064, 10$^{-8}$ mol/L). Pretreatment of quiescent arteries with PGE$_2$, for 5 minutes (10$^{-6}$ mol/L) led to a significant right shift of the concentration–response to norepinephrine (EC$_{50}$ from 6.6±0.1–5.9±0.1). In intrarenal arteries with K$^+$-induced tone, PGE$_2$ and PGI$_2$, at 10$^{-5}$ mol/L elicited increased tension. This was abolished by thromboxane receptor (TP) antagonist (S18886, 10$^{-6}$ mol/L). A TP agonist (U46619, n=6) evoked tension (EC$_{50}$=8.1±0.2) that was inhibited by S18886. Polymerase chain reaction and immunoblotting showed EP4, IP, and TP receptors in intrarenal arteries. In conclusion, PGE$_2$ and PGI$_2$ may protect renal perfusion by activating cognate IP and EP4 receptors associated with smooth muscle cells and endothelium in human intrarenal arteries and contribute to increased renal vascular resistance at high pathological concentrations mediated by noncognate TP receptor. (Hypertension. 2014;64:551-556.)

Key Words: epoprostenol ■ kidney ■ norepinephrine ■ prostaglandin-endoperoxide synthases

In settings with decreased effective circulating volume and in patients with chronically reduced kidney function, renal perfusion and glomerular filtration rate are sensitive to inhibition of prostaglandin synthesis by cyclooxygenase inhibitors. The detrimental effect is attributed predominantly to compromised renal perfusion. An increase in endogenous angiotensin II and norepinephrine in response to extracellular volume contraction is associated with increased urinary excretion of prostaglandin E$_2$ (PGE$_2$), and infusion of angiotensin II and norepinephrine to humans leads to increased excretion of both PGE$_2$ and prostacyclin (PGI$_2$). In experimental animals, the cyclooxygenase inhibitor ibuprofen potentiates angiotensin II–mediated renal preglomerular vasoconstriction. This response is reversed by infusion of low concentrations of PGE$_2$ and restored paradoxically by higher doses of this prostanooid. PGE$_2$ and prostacyclin elicit dilatation of renal pre- and postglomerular resistance vessels. In rodents, the renal vasodilator response to prostaglandin E$_2$ is predominantly attributable to activation of EP4 receptors with a minor contribution from EP2 receptors. EP4 receptors are found in rat and human preglomerular blood vessels and glomeruli. Data on vascular expression of EP2 are less consistent. Prostacyclin receptor (IP) has been detected in human preglomerular blood vessels and vasa recta. Functional data on the role of prostanoid receptors and their reactivity to PGE$_2$ and prostacyclin in human preglomerular vasculature are not available. The present study was designed to test the hypothesis that PGE$_2$ and prostacyclin relax human renal preglomerular arteries through activation of prostanoid receptors (EP2/IP), which when activated lead to increased production of cAMP by adenyl cyclase.

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Materials and Methods

Patient Tissue Collection

Patients were included at Department of Urology and Department of Thoracic and Cardiovascular Surgery at Odense University Hospital, Odense, Denmark. The collection of kidney tissue for artery dissection and left internal thoracic artery (ITA) were approved by the Regional Ethics Committee (kidney: S-VF-20010035; ITA: S-20100044) and was performed only after informed, written consent of the donors. Patient characteristics are shown in Table S1 in the online-only Data Supplement. The dissection procedure was as described previously\(^1\),\(^6\) and further details are found in the online-only Data Supplement.

Protocols, Solution, and Experimental Series

The intrarenal artery rings (median diameter: 362 μm [177; 486]; thickness =2–3 mm, n=46 nephrectomy patients) were suspended for measurement of force generation in a Multi Wire Myograph System, 610M version 2.2 (Danish Myo Technology), filled with Krebs–Henseleit solution aerated with 5% CO\(_2\) in air. Details of microdissection, solutions, agonists, protocols, and experimental series are given in the online-only Data Supplement. To prevent interference from endogenously formed prostanoids, all experiments were performed in the presence of indomethacin (10\(^{-5}\) mol/L), a concentration that did not significantly affect the concentration–response curve to KCl (Figure S1A). K\(^+\) at EC\(_{50}\) (4×10\(^{-2}\) mol/L; Figure S1A) was used to induce stable tension. The response to K\(^+\) at EC\(_{50}\) did not wane for 2 hours and did not display oscillations (Figure S1B). The preparations included were accepted as viable based on a development of force >2 mN in response to KCl. Acetylcholine (10\(^{-5}\) mol/L), added during stable tension, caused relaxation in excess of 10% (of the response to KCl) in 76% of rings (Figure S1C), indicating the presence (or not) of functional endothelium. Responses to agonists, in the absence and presence of pharmacological antagonists, were obtained either in contracted (4×10\(^{-2}\) mol/L KCl) or in quiescent preparations.

Data Analysis and Statistics

Increases in isometric force were expressed relative to either the maximal force (100%) to the respective substance or to KCl (4×10\(^{-2}\) mol/L)-induced tone. Relaxation was expressed as percentage of the preexisting tone. Data are shown as mean±SEM; n represents the number of experiments with rings from different patients. For comparison of single and group mean values, 1-way and 2-way ANOVA followed by Bonferroni multiple comparison tests were performed, respectively, using GraphPad Prism software (GraphPad Prism 5.0). P values <0.05 were considered to indicate statistically significant differences.

Results

Prostaglandin E\(_2\)

The cumulative addition of increasing concentrations (10\(^{-5}\)–10\(^{-3}\) mol/L) of PGE\(_2\) induced concentration-dependent relaxations of intrarenal arteries with an EC\(_{50}\) of 10\(^{-6}\) mol/L (Figure 1A and 1B). At 10\(^{-5}\) mol/L, PGE\(_2\) caused a significant increase in tension (Figure 1A and 1B). Addition of a single concentration (10\(^{-6}\) mol/L) of PGE\(_2\), to K\(^+\)-stimulated intrarenal arteries yielded a significantly larger relaxation than that obtained with the same concentration as part of a cumulative concentration–response (Figure S2A). There was a significantly larger relaxation in response to 10\(^{-4}\) mol/L PGE\(_2\) in arteries with functional endothelium (Figure S2B). In K\(^+\)-stimulated left ITA, PGE\(_2\) (10\(^{-6}\)–10\(^{-5}\) mol/L) caused concentration-dependent increases in tension (Figure S2D).

Norepinephrine caused concentration (10\(^{-5}\)–10\(^{-3}\) mol/L)-dependent increases in tension (EC\(_{50}\)=6.6±0.1 mol/L; Figure 2A). Pretreatment with 10\(^{-6}\) mol/L PGE\(_2\), for 5 minutes before the exposure to norepinephrine caused a significant rightward shift of the concentration–response curve (EC\(_{50}: 5.9±0.1\) mol/L) without affecting the maximal response to the catecholamine (Figure 2A). The selective EP\(_4\) agonist CAY10598\(^17\) (10\(^{-8}\)–10\(^{-6}\) mol/L) induced concentration-dependent relaxations of K\(^+\)-stimulated arteries that was not significantly different from the response to PGE\(_2\) in concentrations ≤10\(^{-6}\) mol/L (Figure 1 versus Figure 2). CAY10598 did not induce tension at higher concentrations but rather decreased tension further (Figure 2B and 2C).

Prostacyclin

Addition of prostacyclin (10\(^{-8}\)–10\(^{-5}\) mol/L) to KCl-stimulated intrarenal arteries yielded concentration-dependent relaxations ≤10\(^{-6}\) mol/L (EC\(_{50}: log 7.7\) mol/L; Figure 3A). Stratification of the arteries according to endothelial function did not yield significant correlation between the responsiveness to prostacyclin and that to acetylcholine (data not shown). At concentrations of prostacyclin >10\(^{-6}\) mol/L, the relaxation reversed to a significant increase in tension (Figure 3A and 3B). The IP\(_3\) receptor antagonist BR5064 (10\(^{-6}\) mol/L) abolished prostacyclin-induced relaxations while increased tension at 10\(^{-5}\) mol/L was not affected by the blocker (Figure 3C). With ITA, prostacyclin elicited a similar relaxation but with a markedly stronger increase in tension at high concentrations (4x above the KCl-induced tone; Figure S4C).
To determine whether or not TP receptors are involved in PGE$_2$ and prostacyclin-induced force generation (Figures 1A and 3A), the selective TP receptor antagonist S18886 (terutroban, $10^{-6}$ mol/L for 5 minutes) was added to the bath solution. S18886 did not affect relaxations but abolished PGE$_2$-induced (Figure 4A) and prostacyclin-induced (Figure 4B) force development (original traces in Figure S3). The TP receptor agonist U46619 caused concentration-dependent increases in tension in intrarenal arteries with EC$_{50}$ values of $(-\log$ mol/L) 7.7±0.2 (Figure S4A).
increased tension of U46619 was antagonized by S18886 at concentrations from 10⁻⁶ mol/L (Figure S4B). In ITA, the increase in tension (in response to the highest concentration of prostacyclin) was abolished by the TP receptor antagonist (Figure S4C).

**EP, IP, and TP Receptor Expression**

By Western immunoblotting of homogenates from intrarenal artery rings from same segments as used for myography, proteins with a migratory pattern at the predicted molecular size of receptors EP4 (52 kDa), IP (deglycosylated protein at 40 kDa), and TP (55 kDa) were observed (Figure 5). Immunostaining of kidney sections showed IP receptor labeling in media smooth muscle of intrarenal arteries (Figure 5). Intrarenal arteries expressed EP4, IP, and TP receptors and PGI₂ and thromboxane A₂ synthases (Figure S5 and S6).

**Discussion**

The present study shows that PGE₂ and prostacyclin exert dual effects on human intrarenal arteries with relaxation in the nanomolar range, mediated by EP4 and IP receptors, whereas in high micromolar concentrations, tension development was observed resulting from thromboxane TP receptor activation. PGE₂ led to a significant right shift in the concentration–response to norepinephrine. Thus, PGE₂ and PGI₂ may account for the support of renal blood flow and glomerular filtration rate that is uncovered by inhibitors of cyclooxygenase in patients with challenged kidney function. The tested artery rings had median diameter of ≈360 μm, which is exactly between human arcuate arteries (4–500 μm) and cortical radial arteries (150–200 μm). There was little variation in concentration–response to K⁺, norepinephrine, prostanoids, and receptor antagonists. This suggests effective washout of drugs in the isolation procedure and a stable expression pattern of voltage-gated calcium channels, prostanoid receptors, and adrenoceptors in intrarenal arteries across age, sex, and morbidities. Acetylcholine-mediated relaxation was observed in 75% of vessels. Because endothelium-derived hyperpolarization is responsible for acetylcholine-induced responses in NO-blocked, human renal interlobar arteries and K⁺-depolarization attenuates the response to acetylcholine compared with agonists, the present data may have underestimated the functional state of the endothelium and the relaxing effect of PGI₂ and prostacyclin because membrane potential is clamped in response to elevated K⁺.

The relaxation induced by prostacyclin was blocked by an IP receptor antagonist; the response was independent of the
functional state of the endothelium, and IP mRNA and protein were detected in intrarenal arteries. This indicates that prostacyclin mediates an IP receptor–dependent relaxation through a direct action on the vascular smooth muscle. Previous studies with systemic infusion of prostacyclin \(^\text{14}\) and iloprost \(^\text{25}\) to healthy humans showed a significant increase in renal plasma flow, despite a decrease in arterial blood pressure. This is in agreement with the present findings of a direct relaxant action by prostacyclin on human renal resistance vessels. PGE\(_2\) in nanomolar concentration relaxed intrarenal arteries. In renal vascular myocytes, PGE\(_2\) attenuates transmembrane calcium influx \(^\text{26}\) and suppresses intracellular calcium mobilization. \(^\text{11,27}\)

The PGE\(_2\)-induced relaxation was likely mediated by the EP4 receptor; the response was mimicked by an EP4 agonist; it exhibited desensitization \(^\text{28,29}\) and depended partially on functional state of the endothelium as is the case in the murine aorta. \(^\text{11}\) In accordance with previous immunohistochemical data, EP4 mRNA and protein were present in intrarenal arteries. \(^\text{13}\) The relaxing response to PGE\(_2\) was not observed in ITA. This could be because of impaired endothelial function or to higher EP1/3 receptor expression. The rightward shift by PGE\(_2\) of the concentration–effect curve for norepinephrine in intrarenal arteries confirms observations on renal blood flow in animal studies, for example. \(^\text{30}\) In vitro studies with isolated human renal arteries showed that norepinephrine-induced contractions depend on extracellular calcium with an \(EC_{\text{max}}\) at 10\(^{-3}\) mol/L. \(^\text{30}\) In smaller human intrarenal arteries, this response was mimicked by an \(\alpha_1\)-adrenoceptor agonist. \(^\text{21,31}\) \(\alpha_1\)-Adrenoceptors are expressed in the human renal artery. \(^\text{22}\) The present findings are consistent with the concept that PGE\(_2\) protects renal perfusion in vivo in settings of extracellular volume contraction. Several animal studies show a concentration-dependent constriction of renal vessels in response to PGE\(_2\), in higher concentration. \(^\text{4,5}\) Therefore, the present study also sought to address the question whether this biphasic effect is because of expression of several classes of EP receptors as, for example, in the rat \(^\text{4}\) or to noncognate activation of thromboxane TP receptors, as observed in other vascular beds. \(^\text{13,32}\)

Endogenous TxA\(_2\) production was abolished by S18883, similarly to what was observed in the intrarenal artery. It is concluded that PGE\(_2\) and prostacyclin relax intrarenal arteries through direct effects on EP4 and IP receptors and that prostacyclin and PGE\(_2\) are low-affinity agonists at the TP receptor across human vascular beds.

**Perspectives**

The relaxant effect of PGE\(_2\) and prostacyclin may preserve human renal perfusion during common conditions with a challenged effective circulating volume. In situations with high local concentrations of several prostaglandins and isoprostanes, for example, endotoxemia, \(^\text{38}\) there could be a converging activation of the TP receptor to elicit ischemic renal failure. A cooperative action of angiotensin II-AT1 and TP receptors to lower renal perfusion has been observed in experimental models. \(^\text{8,30}\) The TP receptor seems as an attractive target to counter ischemic renal failure.

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**Disclosures**

None.

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**Novelty and Significance**

**What Is New?**

- Prostanoids prostaglandin Ej (PGEj) and prostacyclin (PGi2) relax human microdissected intrarenal arteries in nanomolar concentration range through receptors EP4 and IP. At concentrations >10–6 mol/L, PGEj and PGii increase tension through thromboxane receptors.

**What Is Relevant?**

- The clinical use of cyclooxygenase inhibitors is associated with renal adverse effects: decline in renal perfusion and glomerular filtration rate, NaCl retention, and hypertension. The set of data shows that PGEj and PGii may preserve renal perfusion through vasodilator receptors associated with human intrarenal resistance vessels. The contraction by PGEj and PGii at high micromolar concentrations through TP may contribute to ischemic renal failure in, for example, endotoxemia/sepsis with large synthesis of cyclooxygenase products.

**Summary**

Through force recordings in myographs, human intrarenal artery rings display functional expression of PGEj, PGi2, and TxA2 receptors EP4, IP, and TP. Although PGEj and PGii exert relaxation in nanomolar concentrations, they induce force generation through TP and thus are low-affinity TP receptor agonists in high micromolar concentrations. Intrarenal generation of prostanooids may exert dual and concentration-dependent effects directly on the renal resistance arteries.
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Extended Materials and Methods

**Preparation of tissue** After surgical removal, the kidney was transported to Department of Clinical Pathology at Odense University Hospital. In cooperation with a pathologist, a wedge-shaped piece of tissue between two renal pyramids was macroscopically identified, isolated and placed in ice-cold buffer (ATM, see Chemicals and Solutions). Tissue was taken from the part of the kidney consisting of macroscopic normal tissue. Under a stereomicroscope with sharpened forceps, the vessels were dissected free and cleared of connective tissue. Care was taken not to traumatize the vessel wall and endothelium. An artery ring was frozen in liquid nitrogen. The artery rings were stored overnight (~16-20h) in ATM at 4°C. This was done 1) to washout anesthetics and drugs and 2) to standardize start procedure with the very variable time of surgery and delivery of the tissue. Each artery was moved to Physiological Salt Solution (PSS, Chemicals and Solutions), cut into rings of 1-2 mm in length and mounted in the myograph according to manufacturers’ protocol.
After mounting, the vessel rings were slowly heated and left to equilibrate for 60 minutes before normalization was commenced.

Human Left internal thoracic artery (ITA) was obtained from patients undergoing Coronary Artery Bypass Graft (CABG). During the surgery the artery was dissected free and exposed by the surgeon and left with blood flow. Just before removing the artery, the surgeon placed clips on the artery to stop blood flow. A small part of the artery, containing clip and downstream segment, was cut off and as quickly as possible placed in ice-cold buffer, placed in a bucket with ice and transported to the laboratory where it was examined under stereomicroscope. Parts with visible damage were discarded. Furthermore only the vessel segment downstream of the clip was used to avoid pressure damage. From this point and on the handling of the tissue was identical with the handling of the renal arteries as described above.

**Isometric Force Measurements in intrarenal arteries** These experiments were conducted on the Multi Wire Myograph System – 610M version 2.2 (Danish Myo Technology – DMT, Aarhus Denmark). This device contained four individual single vessel myographs. Each unit was made of aluminum and contained a steel chamber with two myograph jaws, a lid with holes and individually controlled aeration and suction. The vessel ring was suspended between two jaws on 40 µm wires. One jaw was connected to a transducer-pin that measured the generated force and transferred the signal to an interface, which then again transferred, to a computer. A computer program, Chart ADinstruments (PowerLab, ADInstruments, Colorado Springs, CO, USA), converted the signal into a force trace (milliNewton, mN) in real time. The second jaw was connected to a micropositioner that allowed regulation of the distance between the jaws and therefore apply appropriate strength. The experiments were performed under isometric conditions, as the length between the jaws was held constant. The wall tension was continuously monitored throughout the experiments for
contractile or relaxant effects of the added chemicals or solutions. During the experiments the temperature was kept constant at 37°C by a temperature plate underneath each chamber. The chamber fluid was constantly equilibrated with 5 % CO₂ in air to maintain pH at ~7.4 as well as to ensure sufficient mixing and movement of the added substances and solutions.

**Chemicals and Solutions** Acetylcholine chloride (ACh, Sigma), phenolamine hydrochloride (PHE, Sigma), 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetic acid (Indomethacin, Sigma), Prostaglandin E₂ (PGE₂, Sigma), Norepinephrine (Sigma), Epoprostenolnatrium (Prostacyclin/Flolan, GlaxoSmithKline, Odense University Hospital-Pharmacy), CAY10598 (EP4-agonist, Cayman Chemical Company), 9-11-Dideoxy-11 α-9 α-epoxymethanoprostaglandin F₂α (U46619/TP-agonist, Sigma). S18886 (TP-antagonist) was a kind gift from Institut de Recherches Servier, Suresnes, France and BR5064 (IP-antagonist) was a kind gift from Drs. Peter Sandner and Andreas Knorr from Bayer Schering Pharma. The stock solutions were made using the following solvents: Pure water: Phentolamine, ACh, S18886; 96 % ethanol: Indomethacin, PGE₂, CAY10598; 0.5 mol/L HCl: norepinephrine.0.1 mol/L NaHCO₃: BR5064; Methyl Acetate: U46619; manufacturer’s solvent (pH 10.2-10.8): epoprostenolnatrium/Flolan (synthetic prostacyclin). The stock solutions were prepared in the highest possible concentration (preferably 10⁻² mol/L).

**Physiological Salt Solution (PSS)** Prior to each experiment the PSS was mixed and indomethacin (10 µmol/L) was added. PSS was pH-equilibrated and throughout the experiments bubbled with 5 % CO₂ in air and maintained at a temperature of 37°C. The composition of the physiological saline solution was as follows, in mmol/L: NaCl 115, NaHCO₃ 25, MgSO₄ 1.2, K₂HPO₄ 2.5, CaCl₂ 1.3, glucose 5.5, and HEPES 10.
Ca$^{2+}$-free Atmospheric minimal essential medium (ATM) The ATM medium was used as buffer during dissection and for storage at 4°C. The composition of the ATM solution was as follows, in mmol/L: NaCl 103, KCl 5.4, NaHCO$_3$ 4.0, NaH$_2$PO$_4$ 1.5, MgSO$_4$ 0.8, glucose 5.1, Na-pyruvate 0.9, Na-isethionic acid 30, HEPES 5.6 and in ml/L: MEM vitamin solution 10 (Sigma M6895), MEM essential amino acid solution 20 (Sigma M5550), and MEM nonessential amino acid solution 10 (Sigma M7145).

Normalization procedure The rings were normalized in accordance to the procedure described by the manufacturer. The normalization process was used to determine the passive stretch (the normalized internal circumference (IC$_1$), where the active force generation was largest and the sensitivity to agonists most effective. This ensures reproducibility both in the individual experiment and between multiple experiments. The procedure consists of stepwise distending the vessels and measuring sets of distance and force (mN). These measurements are converted into values of internal circumference (IC) and wall tension (mN/mm). Using the LaPlace Relation effective pressure (P$_i$) was determined:

$$P_i = \text{walltensio n/}\left(\text{Internal circumference} \cdot 2 \times \pi\right)$$

Using the calculated values of P$_i$ an exponential graph with IC on the x-axis and wall tension on the y-axis was created. An isobar curve representing 100 mmHg (13.3 kPa) was applied to the graph. The point where P$_i$ = 100 mmHg corresponds to the intersection of the exponential curve and the isobar curve. The internal circumference of P$_i$ = 100 mmHg is denoted IC$_{100}$ and can be read of the x-axis. IC$_{100}$ is an approximate of the internal circumference of an artery that is unmounted, fully relaxed and under a transmural pressure of 100 mmHg. IC$_1$ is believed to be the most optimal passive stretch and is found as a fraction of IC$_{100}$. In rat mesenteric arteries this fraction is 0.9 (REF). IC$_1$ can therefore be calculated as:
\[ IC_1 = 0.9 \times IC_{100} \]

When \( IC_1 \) was determined the micropositioner was moved to this position and the artery-ring was left for equilibration for 30 minutes and was then ready for experiments.

**Experimental protocols** Experiments were conducted in the presence of indomethacin (10 µmol/L) to block endogenous COX-activity. When \( K^+ \) was used to induce tension, the vessels were incubated for at least 5 minutes prior to experiments with the reversible nonselective alpha-adrenergic antagonist, phentolamine (10 µmol/L) to exclude potential involvement of nerve-mediated responses to depolarization. Whenever an antagonist, e.g. TP-receptor antagonist (S18886) was used, the incubation-time was always 40 minutes prior to the experiment. When mounted, normalized and equilibrated vessels were challenged with a “standard start”. The standard start included viability check of smooth muscle cells (VSCM) and endothelium in each individual vessel. Viability of VSCM was confirmed by contraction to potassium (40 mmol/L \( K^+ \)) and viability and function of the endothelium was confirmed by a relaxation to acetylcholine (ACh, 1 µmol/L). Vessels were classified as “endothelium-intact” if this procedure yielded loss of at least 10% of \( K^+ \)-induced tone. Responses were either relaxation or no response; in no case was in increased tension in response to Ach 1 µmol/L observed. If a preparation responded with force-development above 1 mN to \( K^+ \)-addition, the VSMC was considered viable and the ring was used. After validation the vessels were washed and left for 30 minutes to equilibrate. Standard washing procedure consisted of a change of physiological saline solution (PSS) every 2 minutes, 6 times in all and a 30 minutes period of equilibration.
**Experimental Series** In experiments using vasoactive substances and compounds, one or more vessel rings were used as time-controls (1:1). These vessels were used to observe any spontaneous changes in force over time.

Intrarenal arteries:

1. **Potassium concentration response**

   The arteries were challenged with four increasing concentrations of potassium (K⁺; 25 – 100 mmol/L) with 5 minutes between two adjacent concentrations. Afterwards EC₅₀ and EC₇₀ values were calculated for later use. This experiment was performed on both human intrarenal arteries and human left internal mammary arteries. This was done in the presence and in the absence of indomethacin during incubation and standard start.

2. **Prostaglandin E₂ concentration- response**

   The arteries were stimulated with K⁺ (EC₇₀). When reaching stable constriction, cumulative increasing concentrations of PGE₂ (10⁻⁹–10⁻⁵ mol/L) were added, with 3 minutes between two adjacent concentrations. This experiment was performed on both human intrarenal arteries and human left internal mammary arteries.

3. **Prostaglandin E₂ concentration-response on pre-stimulated arteries with TP-receptor antagonist**

   Two artery-rings were incubated with a TP-antagonist (S18886, 10 µmol/L) 40 minutes prior to the experiment and two were not. Hereafter the experiment was carried out identical to experiment II. This experiment was performed on human intrarenal arteries.

4. **TP-receptor agonist concentration response**

   The arteries were challenged with four increasing concentrations of the TP-agonist, U46619 (1 nmol/L – 1 µmol/L). A higher concentration was added when the constriction of the prior concentration reached maximum. Afterwards EC₅₀ and EC₇₀ values were calculated for later use.
This experiment was performed on both human intrarenal arteries and human left internal mammary arteries.

V. **TP-receptor antagonist concentration response on TP-agonist stimulated arteries**

The arteries were stimulated with TP-receptor agonist (U46619, EC\textsubscript{70}: 8x10\textsuperscript{-8}mol/L). When reaching stable force, cumulative increasing concentrations of TP-receptor antagonist (S18886, 10\textsuperscript{-8} – 10\textsuperscript{-6} mol/L) were added, with 5 min intervals. This experiment was performed on human intrarenal arteries.

VI. **Prostaglandin E\textsubscript{2} single bolus response on stimulated arteries**

The arteries were stimulated with K\textsuperscript{+} (EC\textsubscript{70}). When reaching stable force, a single concentration of PGE\textsubscript{2} (10\textsuperscript{-6} mol/L) was added. This experiment was performed on human intrarenal arteries.

VII. **Prostaglandin E\textsubscript{2} - EP\textsubscript{4} receptor agonist concentration response**

The arteries were stimulated with K\textsuperscript{+} (EC\textsubscript{70}). When reaching stable tension, cumulative increasing concentrations of an EP\textsubscript{4}-agonist (CAY10598, 10\textsuperscript{-9} – 10\textsuperscript{-5} mol/L) were added, with 3 minutes between two adjacent concentrations. This experiment was performed on human intrarenal arteries.

VIII. **Norepinephrine concentration response with and without prostaglandin E\textsubscript{2}**

Two artery-rings were incubated with PGE\textsubscript{2} (1 µmol/L) 5 minutes prior to the experiment and two were not. Hereafter cumulative increasing concentrations of norepinephrine (NE, 10\textsuperscript{-8} – 10\textsuperscript{-5} mol/L) were added, with 5 minutes between two adjacent concentrations. This experiment was performed on human intrarenal arteries.

IX. **Prostacyclin concentration response**

The arteries were stimulated with K\textsuperscript{+} (EC\textsubscript{70}). When reaching stable force, increasing concentrations of PGI\textsubscript{2} (10\textsuperscript{-8} – 10\textsuperscript{-5} mol/L) were added, with 2 minutes between two adjacent concentrations. Due to low stability of PGI\textsubscript{2} at physiological pH the whole volume of the organ bath was exchanged at every concentration step. This experiment was performed on human intrarenal arteries.
X. **Prostacyclin concentration response with IP$_1$-receptor blocker**

Two artery-rings were incubated with an IP$_1$-antagonist (BR5064, $10^{-8}$ mol/L) 40 minutes prior to the experiment and two were not. Hereafter the experiment was carried out identical to experiment IX.

XI. **Prostacyclin concentration response with TP-receptor blocker**

Two artery-rings were incubated with a TP-receptor antagonist (S18886, $10^{-6}$ mol/L) 40 minutes prior to the experiment and two were not. Hereafter the experiment was carried out identical to experiment IX.

**RT-PCR Analysis** RNA was isolated from single and pooled human intrarenal artery rings of exactly same caliber as rings used for myography and from renal cortex and outer medulla tissue. Isolation was done using Trizol reagent according to manufactures instructions (Invitrogen, Calsbad, CA, USA) and quantified on a nanodrop (Implem, AH-diagnostics). 1 μg total RNA was reverse transcribed using iScript cDNA synthesis kit with random hexamer (BioRad). In subsets of samples, reverse transcriptase was omitted and reaction mix was used as a negative control for DNA contamination. 50 ng cDNA was used as template for DNA amplification with the following primers

EP4

sense: 5’-CGGGATCCGCGAATATCAGACCTC-3’;

antisense: 5’-GGAATTCGTTCAGTGTTTCACTGGG-3’, 145 bp

IP

sense: 5’-GCTGGCCCTCATGACAGTTP-3’;

antisense: 5’-TTGCAGGAAAAGGATGAAGAC-3’, 171 bp,
TP

sense: 5’-AGG TGG AGA TGG CTC AG-3’; antisense: 5’-CGG CGG AAC AGG ATA TAC AC-3’, 220 bp

Ribosomal protein L41 (RPL4)

sense5’-AAGATGAGGCAGAGGTCC-3’; antisense: 5’-TCCAGAATGTCACAGGTCCA-3’, 248 BP)

mPGES-1

sense: 5´- AGTGAGGCTGCGGAAGAAG -3´antisense: 3´- CCAGGAAAAGGAAGGGGTAG - 5´, 149 bp,

PGIS

Sense: 5´- GGTATGCCTTGGAAGTGGGA -3’,antisense 3´- GTCCAGGAGAAGGTTGACAT - 5´, 116 bp

PCR was run for 33 cycles: 30 sec at 95°C, 20 sec at 60°C and 30 sec at 72, followed by 10 min at 72C).

PCR products were separated and visualized on a 2% agarose gel with Gel Red-Nucleic Acid Stain (Biotion) using φX174RF DNA HaellIII fragments as size marker.

**Western immunoblotting** Proteins were extracted from isolated intrarenal arteries from either 1 patient or a pool of 4 patients. Human ITA was homogenized in 50-200 μl homogenization buffer (25 mM imidazol, 0.3 M sucrose, 1 mM EDTA pH 7.2 containing mini complete protease inhibitor cocktail mix (Roche). Proteins were separated under denaturizing and reducing conditions on a 4-15% mini protean TGX gel (Biorad); blotted onto a PVDF membrane, blocked for 1 hour in 5% milk tris-buffered-saline containing 0.05% tween-100; incubated over night at 4°C or 1 hour with
primary antibody EP4 (1:500, Cayman); IP (1:250; Cayman); TP (1:200, Cayman), TxAS (1:1000, Abcam) and β-actin (1:5000, Abcam). After several washes, appropriate HRP-labeled secondary goat anti rabbit antibody was added (1:1000, DAKO) and visualized by western ECL pro kit (Perkin Elmer).
**Supplement table S1.**

**Patient characteristics**

The study included 53 patients subjected to unilateral nephrectomy. The median age was 65 years (range 39; 81). Blank cells indicate that the data was not found/not available or not relevant.

**Abbreviations:**

Gender: Male (M) / Female (F). RCC: Renal Cell Carcinoma. BP: Systolic/diastolic blood pressure before surgery. Renography: % Left kidney / Right kidney, * = the kidney with disease. CCB: Calcium-Channel Blocker. ASA: Acetylsalicylic Acid; NSAID non-steroidal anti-inflammatory drug, AT1-angiotensin II AT1-receptor subtype

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A. Concentration response of intrarenal artery rings to isosmotic addition of K⁺ in increasing concentrations in the absence and presence of indomethacin at 10⁻² mol/L.
B. Original trace showing the stable tension development in response to K⁺ at EC₅₀ in intrarenal rings.
C. Original trace from a single, K⁺-stimulated intrarenal artery ring exposed to acetylcholine at 10⁻⁶ mol/L. Acetylcholine evoked relaxation in 75% of microdissected intrarenal arteries.
A. Effect of single or multiple administrations of PGE$_2$ on EC$_{50}$-K$^+$-stimulated human proximal glomerular arteries. x-axis: PGE$_2$ concentration log(PGE$_2$ mol/L); y-axis: relative tension expressed as % of 40 mmol/L KCl; n = 6. ** = P < 0.01, Single vs. Stepup (2 way-ANOVA, Bonferroni posttest).

B. Stratification of the cumulative concentration-response curve to prostaglandin E2 on the same KCl-prestimulated human intrarenal arteries as in figure 1 according to their response to acetylcholine (n = 13). x-axis: PGE$_2$, log(PGE$_2$ mol/L); y-axis: relative tension expressed as % of 40 mmol/L KCl; n = 10. * = P < 0.05 and ** = P < 0.01, ACh response vs. No ACh response, # = P < 0.01 and ## = P < 0.001, ACh response vs. time control, # = P < 0.05. No ACh response vs. time control (two way-ANOVA, Bonferroni post hoc test).

C. Stratification of the concentration-response curve to prostaglandin E2 on the same KCl-stimulated human intrarenal artery rings as in figure 1 according to sex of the donor. There were no significant difference in the response between male (n=5) and female (n=7) donors.

D. Cumulative concentration-response curve to prostaglandin E2 on KCl-stimulated human internal thoracic artery (ITA). x-axis: PGE$_2$, log(PGE$_2$ mol/L); y-axis: relative tension expressed as % of 25 mmol/L KCl(EC$_{70}$); n = 6. *** = P < 0.001 PGE$_2$ vs. time control (two way-ANOVA, Bonferroni post hoc test).
S3
Original traces showing the cumulative concentration-response curve to PGE$_2$ (upper) and prostacyclin (lower) on K$^+$-stimulated human intrarenal artery rings from the same patient in the absence and presence of a TP-receptor antagonist (S18886, 10$^{-6}$ mol/L). The small transient peaks in the lower diagram are mechanical “noise” caused by total exchange of bath fluid due to the short half life of prostacyclin. Average responses are shown in figure 3 in the manuscript.
S4

A. Cumulative concentration-response to TP-receptor agonist (U46619) on human intrarenal arteries. x-axis: U46619, log(U46619 mol/L); y-axis: relative tension expressed as % of 10^-6 mol/L U46619; n=5.

B. Concentration-response to TP-receptor antagonist (S18886) on U46619-stimulated (EC_{50}) human intrarenal arteries. x-axis: S18886, log(S18886 mol/L); y-axis: relative tension expressed as % of 2.3x10^{-9} mol/L U46619; n=2.

C. Effect of PGI_2 on K^+ stimulated human internal thoracic artery in the absence and presence of the TP-receptor antagonist S-18886, n=2
RT-PCR analysis of total RNA isolated from rings made from microdissected intrarenal arteries similar to those used for myography (IR), kidney outer medulla (OM) and cortex (C). Negative controls were samples with addition of water instead of cDNA (H₂O) and with reaction mix without reverse transcriptase (-RT). At 32-33 cycles of amplification, EP4 RNA was faintly detectable in intrarenal arteries, whereas IP and TP appeared abundantly. RPL41 was a housekeeping gene product present in all samples.
S6

RT-PCR analysis of total RNA isolated from single intrarenal artery rings made from microdissected intrarenal arteries (IR). Negative controls were samples with addition of water instead of cDNA (H₂O) and with reaction mix without reverse transcriptase (-RT). At 33 cycles of amplification, PGE synthase (PGES, 149bp) was faintly detectable in 3 of 8 intrarenal artery rings, whereas PGI synthase (PGIS, 116bp) appeared abundantly. TxA synthase (TxA5) was detected in protein homogenate from human kidney cortex (number are microgram protein loaded) and intrarenal (IR) and Internal Thoracic (MA) artery rings by immunoblotting. Expected molecular size is 61 kDa.