Glomerular Actions of a Free Radical-Generated Novel Prostaglandin, 8-epi-Prostaglandin $F_{2\alpha}$, in the Rat

Evidence for Interaction with Thromboxane A_2 Receptors

Kihito Takahashi,* Tarek M. Nammour,* Megumu Fukunaga,* Joan Ebert,* Jason D. Morrow,§ L. Jackson Roberts II,§ Richard L. Hoover,‡ and Kamal F. Badr*

Departments of Medicine, Pathology,‡ and Pharmacology,§ Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

Abstract

8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and related compounds are novel prostanoid produced by a noncyclooxygenase mechanism involving lipid peroxidation. Renal ischemia-reperfusion injury increased urinary excretion of these compounds by 300% over baseline level. Intrarenal arterial infusion at 0.5, 1, and 2 μg/kg per min induced dose-dependent reductions in glomerular filtration rate (GFR) and renal plasma flow, with renal function ceasing at the highest dose. Micropuncture measurements (0.5 μg/kg per min) revealed a predominant increase in afferent resistance, resulting in a decrease in transcapillary hydraulic pressure difference, and leading to reductions in single nephron GFR and plasma flow. These changes were completely abolished or reversed by a TxA_2 receptor antagonist, SQ 29,548. Competitive radioligand binding studies demonstrated that 8-epi-$PGF_{2\alpha}$ is a potent competitor for [^{3}H]SQ 29,548 binding to rat renal arterial smooth muscle cells (RASM) in culture. Furthermore, addition of 8-epi-$PGF_{2\alpha}$ to RASM or isolated glomeruli was not associated with stimulation of arachidonate cyclooxygenase products. Therefore, 8-epi-$PGF_{2\alpha}$ is a potent preglomerular vasoconstrictor acting principally through TxA_2 receptor activation. These findings may explain, in part, the beneficial effects of antioxidant therapy and TxA_2 antagonism observed in numerous models of renal injury induced by lipid peroxidation. (*J. Clin. Invest.* 1992. 90:136–141.) Key words: glomerulonephritis • free radicals • prostaglandins • thromboxane • renal blood flow

Introduction

The generation and pathophysiologic relevance of reactive oxygen metabolites during glomerular injury is well established (1). We have reported the discovery of a series of novel prostaglandin F_2 (PGF_2)-like compounds produced by a noncyclooxygenase mechanism involving free radical catalyzed lipid peroxidation (2). These compounds are easily detected in normal human plasma and urine, and their production is markedly enhanced in animal models with free radical-induced injury (2). Further, we examined the actions of one of these compounds, 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), in normal rat kidney, and demonstrated extremely potent and selective renal vasoconstrictive effects in the low nanomolar concentration range (2). The fact that these prostanoids are present in urine and their formation increases in situations involving free radical-induced injury, combined with the potent renal constrictor action demonstrated for 8-epi-$PGF_{2\alpha}$, suggests a potential role for this family of eicosanoids as novel mediators of the renal functional deterioration that accompanies free radical-induced glomerular injury (1, 2).

In the present study, using micropuncture techniques, we examined the precise responses of glomerular hemodynamics to intrarenal arterial administration of 8-epi-$PGF_{2\alpha}$ in vivo in the normal rat. In addition, we assessed the role of thromboxane receptors in mediating its renal effects both in vivo and in vitro. Furthermore, to investigate the pathophysiologic relevance of these compounds, we assessed their urinary excretion rate in ischemic acute tubular necrosis, an oxygen free radical-mediated experimental model of renal injury (3, 4).

Methods

8-epi-$PGF_{2\alpha}$ (gift from Dr. Gordon Bundy, The Upjohn Co., Kalamazoo, MI). Aliquots of 8-epi-$PGF_{2\alpha}$ were dissolved in ethanol and were stored at −70°C. Immediately before use, they were diluted with 0.1 N PBS to the required concentration. Eicosanoid purity and concentration in stock solutions and in syringes used for intrarenal injections were verified periodically by gas chromatography/negative ion mass spectrometry as previously described (5).

Physiological measurements. Clearance studies were performed on anesthetized adult male Munich-Wistar rats weighing 200–230 g prepared according to the protocols described previously (6). In brief, after Inactin anesthesia (Andrew Lockwood & Associates, Sturtevant, WI), the left femoral artery catheter was used to sample blood and to monitor mean systemic arterial pressure (MAP)[1] by means of a pressure transducer (model P23Db, Statham Instrument; Oxnard, CA) connected to a direct writing recorder (Gould Inc. Instruments Div., Cleveland, OH). After a tracheostomy, PE-50 catheters were inserted into both jugular veins for infusions of plasma, inulin, and paraaminohip-

This work was presented in abstract form at the 23rd Annual Meeting of the American Society of Nephrology, 2–5 December 1990, Washington, DC.

Address correspondence to Kihito Takahashi, M.D., S-3223 Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232-2372.

Received for publication 4 September 1991 and in revised form 30 December 1991

J. Clin. Invest.

0021-9738/92/07/0136/06 $2.00
Volume 90, July 1992, 136–141

1. *Abbreviations used in this paper:* FF, filtration fraction; GFR, glomerular filtration rate; Hct, hematocrit; K_f, glomerular capillary ultrafiltration coefficient; MAP, systemic arterial pressure; PAH, paraaminohippuric acid; P_E, efferent arteriolar pressure; P_{GC}, glomerular capillary pressure; P_T, proximal tubular pressure; R_A, afferent arteriolar resistance; RASM, renal arterial vascular smooth muscle cells; RBF, renal blood flow; RPF, renal plasma flow; R_E, efferent arteriolar resistance; SNGFR, single nephron glomerular filtration rate; SNFF, single nephron filtration fraction; SNPF, single nephron glomerular plasma; V, urine flow rate.

puric acid (PAH) (7.5% and 1.6% solution, respectively, in 0.9% NaCl at 1.2 ml/h), and other experimental solutions. The left kidney was exposed by a left subcostal incision, separated from the surrounding fat, and suspended on a Lucite holder. The kidney surface was bathed with warm isotonic NaCl. A 30-gauge needle was placed in the abdominal aorta at the takeoff of the left renal artery, through which a maintenance infusion of 0.9% NaCl was initiated at a rate of 0.025 ml/min. Homologous rat plasma was administered intravenously at a rate of 10 ml/kg per h for 45 min, followed by a reduction in an infusion rate to 1.5 ml/kg per h for the remainder of the experiment. Previously, this protocol has been shown to adequately replace surgically induced plasma losses, thus maintaining euvolemia (7). In all experiments, measurements were started 60 min after the onset of plasma infusion and carried out as follows: one to three samples of urine from the experimental kidney were collected, each over 10 min, for the determination of urine flow rate (V), inulin and PAH concentrations, and for the calculation of whole kidney glomerular filtration rate (GFR) and renal plasma flow (RPF). For these urine collections, indwelling ureteral catheters (PE 10) were used. Coincident with these urine collections, two or three samples of femoral arterial blood were obtained in each period for determination of hematocrit (Hct) and plasma concentration of inulin and PAH. Whole kidney inulin and PAH clearances were performed during baseline conditions and repeated during administration of 8-epi-$PGF_{2\alpha}$ and other experimental conditions to be described. Inulin concentrations in plasma and urine were determined by the macroanthrone method (8). PAH concentrations in urine and plasma were determined according to the method of Smith et al. (9).

Experiments were performed on four groups of rats as follows.

Group I: time control (n = *7*). In this group, baseline measurements were performed and repeated during a 30-min infusion of vehicle (0.1 N PBS).

Group II: intrarenal arterial 8-epi-$PGF_{2\alpha}$ dose response (n = *21*). In this group, whole kidney measurements were performed during baseline conditions and during the infusion of 8-epi-$PGF_{2\alpha}$ in the following doses: Group IIA: 0.5 μg/kg/min (n = 7); group IIB: 1 μg/kg/min (n = 7); group IIC: 2 μg/kg/min (n = 7). In our previous study (2), we demonstrated that the plasma concentration of 8-epi-$PGF_{2\alpha}$ 30 min after the initiation of a 5-μg/kg per min intravenous infusion was 34 nM (~ 1 ng/ml). This concentration is equivalent to that which is observed in free-radical related injuries in rats (CCl_4-induced liver injury and diquat treatment in selenium deficiency). In our current study, we chose the doses described above, which theoretically deliver similar intrarenal concentrations of 8-epi-$PGF_{2\alpha}$.

Since the experimental period in group II was associated with a time-dependent severe and progressive reduction in renal perfusion, measurements were performed in one 15-min period.

Group III: intrarenal arterial infusion of 8-epi-$PGF_{2\alpha}$ in the presence of a thromboxane A_2 (TxA_2) receptor antagonist SQ 29,548 (10, 11) (n = *7*). In this group, 8-epi-$PGF_{2\alpha}$ was administered at a rate of 2 μg/kg per min (n = 7) in the presence of TxA_2 receptor antagonist SQ 29,548 administered by a sustained infusion at 3 mg/kg per h. Treatment with SQ 29,548 was started 30 min before the initial baseline measurements. Additionally, three rats in group IIC received SQ 29,548 (3 mg/kg per h) 15 min after the initiation of 8-epi-$PGF_{2\alpha}$ infusion (2 μg/kg per min), an experimental period during which measurements were performed in the presence of 8-epi-$PGF_{2\alpha}$ alone. For continuous monitoring of renal blood flow (RBF), an electromagnetic flow probe was placed around the left renal artery and connected to a flow meter (Carolina Medical Electronics, King, NC).

Group IV (n = *7*): *micropuncture measurements during 8-epi-$PGF_{2\alpha}$ infusion.* In this group, glomerular micropuncture measurements were performed during baseline conditions and repeated during 8-epi-$PGF_{2\alpha}$ administration at 0.5 μg/kg per min.

All micropuncture experiments were performed on anesthetized adult male Munich-Wistar rats that weighed 200–225 g and were prepared for micropuncture according to the protocols described previously (12). The preparation is identical to that described above for whole kidney clearance studies, except that ^{3}H-inulin (300 μCi/experimental period in 0.9% NaCl at 1.2 ml per h) from New England Nuclear (Boston, MA) was used instead of nonradiolabeled inulin. Micropuncture measurements were started 60 min after the onset of plasma infusion and carried out as follows: 2-min samples of fluid were collected from surface proximal convolutions to determine the flow rate and inulin concentration. Concomitantly, femoral arterial blood samples were obtained during each period to determine Hct and plasma protein and inulin concentrations. Three samples of blood were obtained from surface efferent arterioles (star vessels) to determine efferent arteriolar protein concentration. Time-averaged hydraulic pressures were measured in surface glomerular capillaries (P_{GC}), proximal tubules (P_T), and surface efferent arterioles (P_E) using a servo-null micropipette transducer system (model 5; Instrumentation for Physiology & Medicine, Inc., San Diego, CA) and micropipettes with outer tip diameters of 2–3 μm containing 2 M NaCl. Colloid osmotic pressures of plasma entering and leaving glomerular capillaries, single nephron glomerular filtration rate (SNGFR), single nephron filtration fraction (SNFF), glomerular capillary ultrafiltration coefficient (K_f), resistances of single afferent (R_A) and efferent (R_E) arterioles, and single nephron glomerular capillary plasma flow (SNPF) were determined using equations described in detail elsewhere (13). The concentrations of inulin in tubular fluid and plasma were determined by measuring the radioactivity of ^{3}H-inulin in a scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Protein concentrations in efferent arteriolar and femoral arterial blood plasmas were determined using a fluorometric method developed by Viets et al. (14).

Group V (n = *4*): *ischemic acute tubular necrosis model.* In this group, urine was collected from the catheter in the left ureter for 15 min under baseline conditions and the left renal artery was clamped for 30 min. 20 min after relief of the clamp from the renal artery, urine was again collected for 15 min. $PGF_{2\alpha}$-compounds in these samples was analyzed after thin layer chromatography purification by gas chromatography/negative ion mass spectrometry as described in (5), and the urinary excretion rate of $PGF_{2\alpha}$-compounds was expressed in pg/min.

Eicosanoids production by 8-epi-$PGF_{2\alpha}$ from isolated glomeruli and rat renal arterial vascular smooth muscle cells (RASM). Glomeruli were isolated from rat kidneys as described previously (15). RASM were cultured from medial explants of rat main renal arteries by the modified method of Ross (16). The resulting cells were grown in RPMI 1640 medium with 10% fasting blood sugar, penicillin (100 U/ml) and streptomycin (100 μg/ml) from Gibco Laboratories, Gibco Div. (St. Lawrence, MA), plated into 100 mm cell culture petri dishes, and incubated at 37°C in a humidified atmosphere of 95% O_2/5% CO_2. Smooth muscle cell colonies were subcultured in 60 mm culture dishes, and experiments were carried out on cells from passages 6–12.

The isolated glomeruli (3,000 glomeruli per incubation) and RASM (10^6 cells per incubation) were incubated in RPMI 1640 maintained at 37°C and 5% CO_2 for 15 min in the absence or presence of increasing amount of 8-epi-$PGF_{2\alpha}$ (10^{-9} to 10^{-6} M). The preparation was centrifuged and the supernatant was frozen at −70°C for TxA_2, prostaglandin I_2 (PGI_2) or prostaglandin E_2 (PGE_2) assay at a later time. The amount of TxB_2, 6-keto-$PGF_{1\alpha}$, the stable metabolites of TxA_2, PGI_2, respectively, and PGE_2 in the supernatants were determined by radioimmunoassay (Amersham Corp., Arlington Heights, IL).

Binding studies. Studies of [^{3}H]SQ 29,548 binding were performed on RASM grown to confluence in 24-well cluster dishes as described elsewhere (17, 18).

Cells were washed once with PBS and then exposed to the appropriate concentration of [^{3}H]SQ 29,548 in Hanks' medium, pH 7.6, containing 0.1% bovine serum albumin. At the completion of the experiment, the experimental medium was removed and the cells were washed five times with ice-cold PBS. The cells were then dissolved with 1 ml of N NaOH, neutralized with HCl, dissolved in 10 ml of Aquasol (New England Nuclear, Boston, MA), and the bound radioactivity was determined using a scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Nonspecific binding was determined by measuring the amount of [^{3}H]SQ 29,548 bound in the presence of 1,000-fold excess of

unlabeled SQ 29,548. Cell density was determined by counting cells from replicate wells, using a Coulter Counter (model ZBi, Coulter Electronics Inc., Hialeah, FL).

The time course of specific [^{3}H]SQ 29,548 binding to RASM was assessed in three experiments in which 5 nM [^{3}H]SQ 29,548 was incubated with RASM as described above and specific binding assessed at 1 min to 24 h after addition of agonist.

Competitive binding-inhibition studies were carried out by incubation of RASM with 5 nM [^{3}H]SQ 29,548 and addition, at equilibrium, of increasing concentrations of 8-epi-$PGF_{2\alpha}$ or unlabeled SQ 29,548.

Statistical analyses. Student's paired *t* test was performed to compare within each group the changes in various whole kidney and microcirculatory indices that occurred from the baseline to the experimental periods. Intergroup multiple comparisons were performed with a one-way analysis of variance followed by the Newman-Keuls test. Differences were considered significant at $P < 0.05$. All values are reported as means±SE.

Results

In vivo studies. Values reported in this section represent the means of repeated measurements performed during the baseline and experimental periods, unless otherwise stated in Methods. The micropuncture values reported are the means of repeated measurements performed early and late in the course of observed responses.

In group I animals (time controls), administration of 8-epi-$PGF_{2\alpha}$ vehicle for a 30-min period was not associated with significant changes in any of the physiologic parameters monitored. Changes in Hct, MAP, GFR, and RPF from the baseline to the experimental period were: Hct, 48.1±0.6 to 47.5±0.6 vol% (NS); MAP, 108±3 to 106±4 mmHg (NS); GFR, 0.93±0.07 to 0.92±0.07 ml/min (NS), which is correspondent to 1.3±1.2% fall; and RPF, 4.44±0.29 to 4.38±0.32 ml/min (NS) (1.6±2.2%). In previous studies, it was shown that repeated measurements of single nephron functions do not reveal any significant changes over this period of time in the presence of stable systemic and whole kidney hemodynamics (19).

In group II rats, administration of increasing doses of intrarenal arterial 8-epi-$PGF_{2\alpha}$ infusion was not associated with significant changes in Hct and MAP, except for a slight increase in group IIC animals. Hct changed from 46.7±0.6 to 46.4±0.5 vol% in group IIA (NS), from 45.8±0.7 to 45.5±0.6 vol% in group IIB (NS), and from 46.8±0.5 to 46.5±0.3 vol% in group IIC (NS). MAP changed from 99±3 to 98±3 mmHg in group IIA (NS), from 107±4 to 109±4 mmHg in group IIB (NS), and from 106±3 to 112±4 mmHg in group IIC ($P < 0.01$). Despite the absence of marked changes in these systemic parameters, the administration of 8-epi-$PGF_{2\alpha}$ was associated with significant and dose-dependent reductions in GFR and RPF (Fig. 1). GFR fell from 0.98±0.07 to 0.69±0.09 ml/min ($P < 0.0005$), which is correspondent to 25.8±3.0% fall in groups IIA; from 1±0.05 to 0.51±0.04 ml/min ($P < 0.0005$) (49.1±4.1% fall) in group IIB; from 0.95±0.04 to 0.27±0.05 ml/min ($P < 0.0005$) (72.4±5.1% fall) in group IIC. RPF fell from 4.43±0.36 to 3.46±0.31 ml/min ($P < 0.005$) (20.8±3.4% fall) in group IIA; from 4.85±0.39 to 2.89±0.41 ml/min ($P < 0.0005$) (43.3±4.7% fall) in group IIB; from 4.83±0.16 to 1.76±0.29 ml/min ($P < 0.0005$) (62.4±6.0% fall) in group IIC. A proportionately greater decrease in GFR compared to RPF resulted in a fall in filtration fraction (FF) that was not statistically significant at the lower dose of 8-epi-$PGF_{2\alpha}$ (groups IIA and IIB, in which FF changed from 0.23±0.03 to 0.21±0.03, NS, and 0.19±0.01 to 0.17±0.02, NS respectively), but was significant at the maximum dose in IIC (0.20±0.01 to 0.15±0.01, $P < 0.025$).

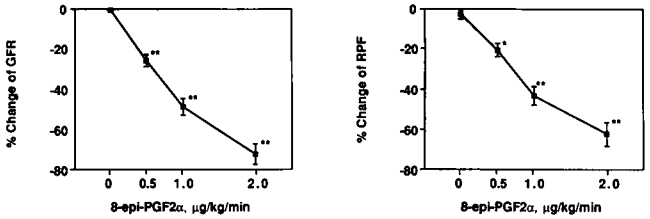

Figure 1. Dose-dependent reductions in GFR and RPF caused by intrarenal arterial infusion of 8-epi-$PGF_{2\alpha}$ at a rate of 0.5, 1, and 2 μg/kg/min. Asterisks indicate significant difference from values observed in vehicle-infused rats as shown 0 μg/kg/min (*$P < 0.005$, **$P < 0.0001$).

Examination of renal responses to this compound at the single-nephron level in group IV (Fig. 2) revealed that the decreases in whole kidney GFR and RPF were accompanied by parallel reductions in SNGFR and SNPF, the former falling from 47.1±2.2 to 34.8±2.5 nl/min ($P < 0.0005$), and the latter fell from 204.3±12.6 to 146.3±11.7 nl/min ($P < 0.0005$). The change in FF was not significant (from 0.25±0.01 to 0.24±0.01 (NS). Accompanying these reductions in SNGFR and SNPF were simultaneous 8-epi-$PGF_{2\alpha}$–induced augmentations of both R_A and R_E, which increased from 1.09±0.11 to 1.79±0.19 10^{10}dyn·s·cm^{-5} ($P < 0.0005$), and from 0.80±0.09 to 1.11±0.11 10^{10}dyn·s·cm^{-5} ($P < 0.0005$), respectively. The proportionately greater increase in R_A led to a significant reduction in P_{GC}, which decreased from 45.3±0.4 to 40.1±0.7 mmHg ($P < 0.0005$). P_T also fell slightly during 8-epi-$PGF_{2\alpha}$ administration, from 13±1 to 11±1 mmHg ($P < 0.05$). Mean net transcapillary hydraulic pressure difference (ΔP) was reduced significantly from 33.6±0.8 to 27.7±0.5 mmHg ($P < 0.005$). The presence of filtration pressure disequilibrium during 8-epi-$PGF_{2\alpha}$ administration allowed for the calculation of unique values for K_f in all the experimental animals. The administration of 8-epi-$PGF_{2\alpha}$ was not associated with a significant change in the mean value for this parameter, which changed from 0.064±0.010 to 0.064±0.009 nl/(s·mmHg) (NS).

When 8-epi-$PGF_{2\alpha}$ was administered in the presence of a specific TxA_2 receptor antagonist, SQ 29,548, a marked modification of the former's renal actions was noted. In the presence of SQ 29,548, 8-epi-$PGF_{2\alpha}$ administration was without a significant effect on either Hct or MAP. Hct changed from 46.5±0.8 to 46.3±0.7 vol% (NS), and MAP changed from 103±4 to 105±4 mmHg (NS). In contrast to the effect of 8-epi-$PGF_{2\alpha}$ infusion alone (group IIC), however, 8-epi-$PGF_{2\alpha}$ administration (2 μg/kg per min) in the presence of SQ 29,548 was not associated with any significant changes in GFR and RPF. GFR changed from 0.92±0.05 to 0.89±0.04 ml/min (1.8±5.9% fall), and RPF changed from 4.85±0.14 to 5.07±0.22 ml/min (3.2±3.9% increase (Fig. 3). FF was unchanged between the control (0.19±0.01) and the 8-epi-$PGF_{2\alpha}$ infusion period (0.18±0.01) (NS). Additionally, baseline measurements in this group (i.e. measurements performed in the presence of SQ 29,548, but before the initiation of 8-epi-$PGF_{2\alpha}$ infusion) were

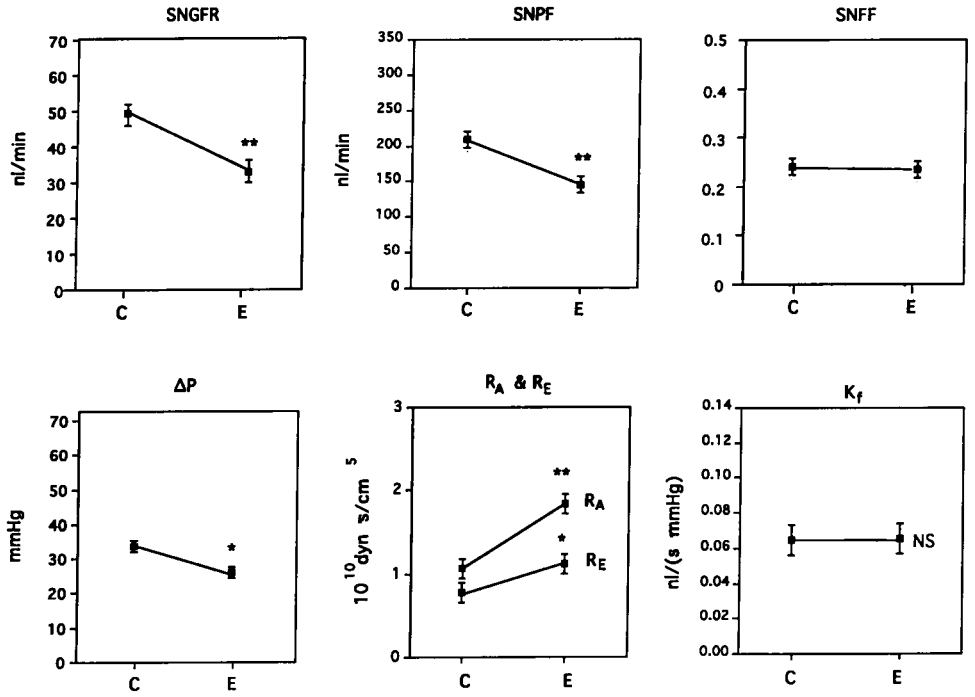

Figure 2. Summary of the changes in glomerular microcirculation before (*C*) and after (*E*) the administration of 8-epi-$PGF_{2\alpha}$ at a rate of 0.5 μg/kg/min. Asterisks indicate significant difference from baseline values (*$P < 0.005$, **$P < 0.0005$).

not significantly different from those observed in groups I and II, suggesting lack of an independent effect for the TxA_2 receptor antagonist on renal or systemic hemodynamics. In addition, three rats in group IIC received SQ 29,548 (3 mg/kg per h) concomitantly with 8-epi-$PGF_{2\alpha}$ (2 μg/kg per min), after a 15-min experimental period with 8-epi-$PGF_{2\alpha}$ alone. The reductions in GFR (0.22±0.01, compared to 0.92±0.08 ml/min in control period, $P < 0.005$) and RPF (1.59±0.13, compared with 4.74±0.26 ml/min in control period, $P < 0.005$) induced by 8-epi-$PGF_{2\alpha}$ were completely reversed to baseline level (GFR: 0.87±0.05 ml/min, NS vs. baseline value; RPF: 4.74±0.26 ml/min, NS vs. baseline value). FF, reduced by 8-epi-$PGF_{2\alpha}$ (0.14±0.01 vs. 0.19±0.01 in control period, $P < 0.05$), also returned to baseline level (0.19±0.01, NS vs. baseline value). Fig. 4 illustrates a representative tracing of RBF monitoring from this group. In the four other animals in group IIC, GFR and RPF deteriorated to naught 30 min after starting 8-epi-$PGF_{2\alpha}$ infusion. Of note, the slightly elevated MAP (116±4 vs. 113±4 mmHg in control period, $P < 0.025$) was also reduced to baseline level (111±4 mmHg, NS vs. baseline value).

Additionally, the presence of a cyclooxygenase inhibitor, ibuprofen (2 mg/kg i.v.), had no effect on the renal responses to 8-epi-$PGF_{2\alpha}$ (2 μg/kg per min) observed in group IIC animals described above (data not shown).

In group V animals, the urinary excretion rates of $PGF_{2\alpha}$ compounds increased from 21.6±1.4 under basal condition to 68.2±47.6 pg/min ($P < 0.05$) during the reperfusion period (Fig. 5).

In vitro studies

Secondary release of eicosanoids by 8-epi-$PGF_{2\alpha}$. The calculated cross-reactivity to 8-epi-$PGF_{2\alpha}$ of the antibody for TxB_2 was 0.5%. The results obtained from the experiments were

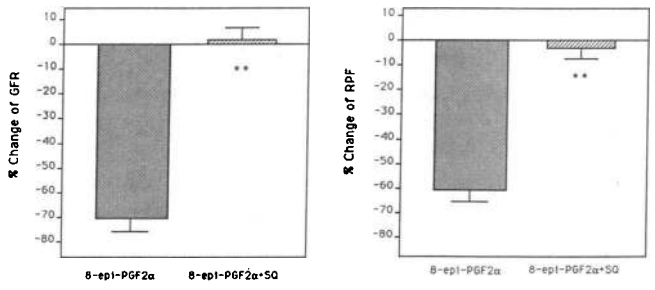

Figure 3. Effects of a thromboxane receptor antagonist on the renal actions of 8-epi-$PGF_{2\alpha}$. In the presence of SQ 29,548 (3 mg/kg per h), the reductions in GFR and RPF caused by 2 μg/kg/min of 8-epi-$PGF_{2\alpha}$ were abolished. Asterisks (**) indicate significant difference ($P < 0.0001$) between changes caused by 8-epi-$PGF_{2\alpha}$ with and without SQ 29,548.

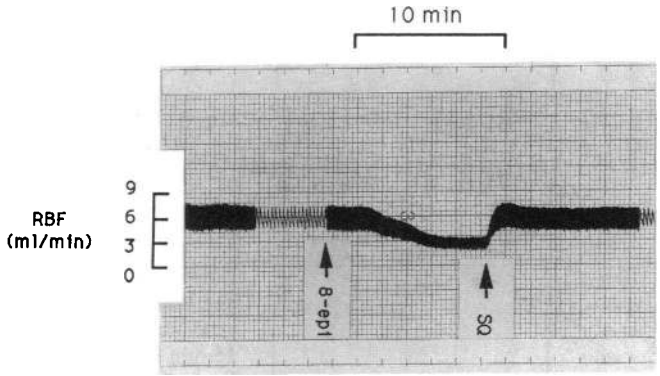

Figure 4. Effect of SQ 29,548 (3 mg/kg per h) on the decreasing RBF by continuous infusion of 8-epi-$PGF_{2\alpha}$ (2 μg/kg/min). The decreasing RBF by 8-epi-$PGF_{2\alpha}$ (8-epi indicates the start of 8-epi-$PGF_{2\alpha}$ infusion) was immediately reversed by the initiation of concomitant SQ 29,548 infusion (indicated by SQ).

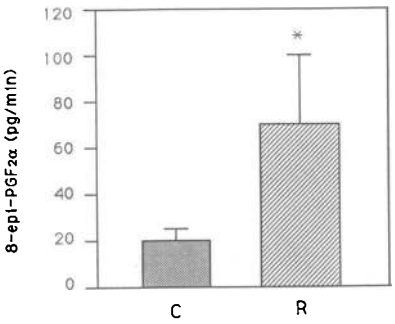

Figure 5. Urinary excretion rate of $PGF_{2\alpha}$-compounds, indicated as 8-epi-$PGF_{2\alpha}$ (pg/min) during control (column *C*) and after release of renal artery clamp (column *R*). An asterisk indicates significant difference from control values (*$P < 0.05$).

corrected based on this cross-reactivity. The cross-reactivities of the antibodies for PGE_2 and 6-keto-$PGF_{1\alpha}$ were negligible. As shown in Fig. 6, incubation of isolated glomeruli with increasing amounts of 8-epi-$PGF_{2\alpha}$ was not associated with significant changes in the amount of TxB_2 (Fig. 6 *A*) and PGE_2 (Fig. 6 *B*) in the supernatant. No significant change was again noted in the value for 6-keto-$PGF_{1\alpha}$ (Fig. 6 *C*) in the supernatant of RASM incubated with increasing concentrations of 8-epi-$PGF_{2\alpha}$. TxB_2 was undetectable in the RASM supernatant incubated even with the highest concentration of 8-epi-$PGF_{2\alpha}$ (10^{-5} M).

Binding study. As shown in Fig. 7 *A*, specific binding of 5 nM [^{3}H]SQ 29,548 to RASM increased progressively with time, reaching a maximum at 6 h and remaining stable for up to 24 h. The properties of SQ 29,548 binding sites on RASM with a dissociation constant (K_d) of 43 nM and specific binding sites (B_{max}) of 2.1×10^5 sites/cell agreed with those observed in the studies using rat aortic smooth muscle cells reported previously (17, 18). Fig. 7 *B* displays the result of competitive inhibition studies. Unlabeled 8-epi-$PGF_{2\alpha}$ and SQ 29,548 competed for the specific binding of [^{3}H]SQ 29,548 to RASM in a concentration-dependent manner and with equivalent potency. Half-maximal inhibition of binding by 8-epi-$PGF_{2\alpha}$ was at 100 nM.

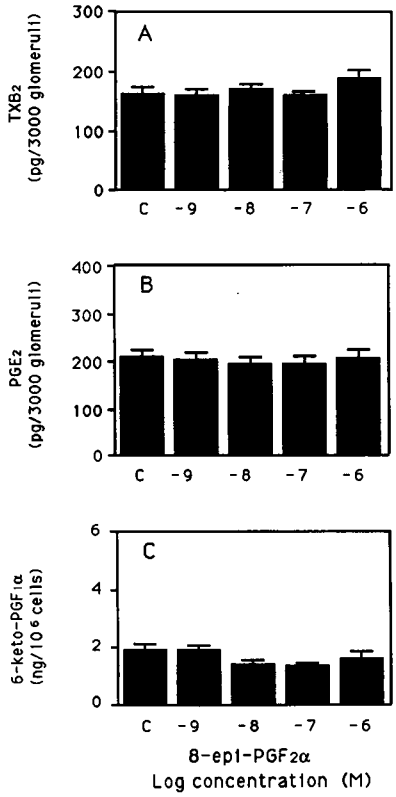

Figure 6. Glomerular and RASM eicosanoids generation following 15-min incubation of increasing amounts of 8-epi-$PGF_{2\alpha}$. (*A*) Glomerular PGE_2 generation. (*B*) Glomerular TxA_2 generation. (*C*) RASM PGI_2 generation.

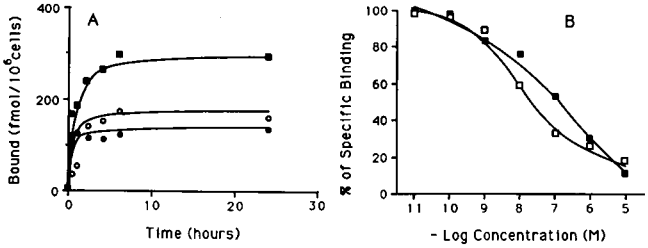

Figure 7. Receptor binding studies. (*A*) Saturation binding of [^{3}H]SQ 29,548 to RASM. *Closed square:* total binding. *Closed circle:* nonspecific binding. *Open circle:* specific binding. (*B*) Competitive inhibition of specific binding of [^{3}H]SQ 29,548 to RASM by 8-epi-$PGF_{2\alpha}$ and non-labelled SQ 29,548. The control value (100%) was defined as the specific binding of [^{3}H]SQ 29,548 in the absence of these compounds. Each point is the mean of triplicate measurements in a typical experiment. *Closed square:* 8-epi-$PGF_{2\alpha}$. *Open square:* SQ 29,548.

Discussion

The present experiments confirmed that intrarenal arterial administration of 8-epi-$PGF_{2\alpha}$ exerted dose-dependent reductions of GFR and RPF. This was associated with only minimal changes in MAP at the highest dose. Of note, a predominant effect of this compound on GFR, as compared to RPF, was observed as the administered dose was increased: this was evidenced by a significant fall in FF in group IIC (2 μg/kg per min). It is interesting to note that the inversion of the stereochemistry at C-8 renders 8-epi-$PGF_{2\alpha}$, a potent renal vasoconstrictor, as compared to $PGF_{2\alpha}$, which, at similar doses, has minimal effect, if any, on renal vascular tone in the rat (20, 21).

To identify the precise effector loci of this prostanoid, we performed micropuncture measurements only at the lowest dose (0.5 μg/kg per min), because severe progressive vasoconstriction induced by higher doses of this compound prevented the performance of reliable micropuncture measurements. This examination revealed a predominant augmentation of R_A, leading to reductions in SNPF and P_{GC}. Consequently, the 8-epi-$PGF_{2\alpha}$-induced fall in GFR at a low dose, was most likely because of a fall in SNPF, as evidenced by insignificant changes in both FF and K_f. The reduction of GFR at a high dose of 8-epi-$PGF_{2\alpha}$ (2 μg/kg per min), however, is likely to result from another mechanism, probably the fall in K_f, in addition to the fall in plasma flow (simple vasoconstriction, in the light of predominant effects of this eicosanoid on GFR at higher doses, as evidenced by the fall in FF in group IIC).

Interestingly, the presence of the specific thromboxane receptor antagonist was associated with complete abolition of the reductions in GFR, RPF, and FF, and the increase in MAP observed during 8-epi-$PGF_{2\alpha}$ infusion alone (2 μg/kg per min). These results indicate strongly the involvement of thromboxane receptors in the mechanism(s) by which this prostanoid reduces renal perfusion and GFR. In the light of the high selectivity of TxA_2 receptor to SQ 29,548 (10), it is not likely that other PG receptors play a major role in mediating the hemodynamic effects of 8-epi-$PGF_{2\alpha}$. The participation of thromboxane receptors in the latter mechanism, coupled with the pre-

vious demonstration of the capacity of TxA_2 to contract mesangial cells (22), and the improvement by thromboxane receptor blockade of low K_f values in previous studies (15, 23), supports the hypothesis that 8-epi-$PGF_{2\alpha}$–induced reductions in GFR at higher doses may be partly because of a significant fall in K_f, an effect thought to be mediated through reductions in glomerular capillary surface area secondary to contraction of mesangial cells (24). Further, subsequent administration of SQ 29,548 during continuous infusion of 8-epi-$PGF_{2\alpha}$ was associated with prompt and complete normalizations of GFR, RPF, FF, and MAP. This observation, interpreted as the displacement by a thromboxane receptor antagonist of 8-epi-$PGF_{2\alpha}$ from thromboxane receptors on vascular smooth muscle and probably on mesangial cells, argues additionally in favor of the participation of thromboxane receptors in mediating renal and systemic vasoconstriction exerted by 8-epi-$PGF_{2\alpha}$.

To assess this hypothesis, we performed the competitive binding study using vascular smooth muscle cells from rat renal artery (RASM). 8-epi-$PGF_{2\alpha}$ was a potent competitor for [^{3}H]SQ 29,548 binding in a manner similar to unlabeled SQ 29,548 (Fig. 7 *B*). This result, coupled with the failure of 8-epi-$PGF_{2\alpha}$ to stimulate secondary release of arachidonate cyclooxygenase products from isolated glomeruli and RASM (Fig. 6), strongly supports the in vivo observations, suggesting that direct interaction of 8-epi-$PGF_{2\alpha}$ with TxA_2 receptors is the principal mechanism underlying its renal constrictor actions.

The formation of these prostanoids catalyzed by free radicals (2), coupled with the potent renal vascular activity demonstrated for 8-epi-$PGF_{2\alpha}$ at pathologically relevant doses in the present study, suggests a potential pathophysiological role for those compounds as novel mediators in oxidant-related renal injury. In fact, our data demonstrated that during the reperfusion period of ischemic acute tubular necrosis, an oxygen free radical-mediated experimental model of renal injury (3, 4), urinary excretion rate of these eicosanoids increased by more than 300%.

In summary, we examined the responses of the glomerular microcirculation in the rat to 8-epi-$PGF_{2\alpha}$, a novel noncyclooxygenase-derived prostanoid produced as a result of free radical catalyzed lipid peroxidation. Our experiments demonstrate that the primary sites of action of 8-epi-$PGF_{2\alpha}$ are in preglomerular and possibly mesangial smooth muscle, where it exerts potent constrictor effects. These actions result in reductions in renal perfusion and probably in glomerular capillary surface area, thereby depressing GFR. We have also sown that in rat renal circulation, the constrictor effects of 8-epi-$PGF_{2\alpha}$ are caused by its activation of thromboxane receptors. These observations, coupled with the increased production of these prostanoids in certain oxygen free radical-related experimental models, suggest a pathophysiological role for free radical-generated prostaglandins in mediating the renal functional derangements observed in oxidant-related renal injury. In addition, our findings might provide an explanation for the salutary effects of TxA_2 antagonism demonstrated in models of lipid peroxidation-induced renal pathophysiology (25).

Acknowledgments

This work was supported by National Institutes of Health grants DK 39261, DK 43883, and GM 42056. Jason D. Morrow is a Howard Hughs Medical Institute Physician Research Fellow.

References

1. Shah, S. V. 1991. Oxidant mechanisms in glomerulonephritis. *Semin. Nephrol.* 11:320–326.

2. Morrow, J. D., K. E. Hill, R. F. Burk, T. M. Nammour, K. F. Badr, and L. J. Roberts, II. 1990. A series of prostaglandin F_2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc. Natl. Acad. Sci. USA.* 87:9383–9387.

3. Hansson, R., O. Jonsson, S. Lundstam, S. Pettersson, T. Schersten, and J. Waldenstrom. 1983. Effects of free radical scavengers on renal circulation after ischemia in the rabbit. *Clin. Sci. (Lond.).* 65:605–610.

4. Paller, M. S., J. R. Hoidal, and T. F. Ferris. 1984. Oxygen free radicals in ischemic acute renal failure in the rat. *J. Clin. Invest.* 74:1156–1164.

5. Morrow, J. D., T. M. Harris, and L. J. Roberts II. 1990. Noncyclooxygenase oxidative formation of a series of novel prostaglandins: analytical ramifications for measurement of eicosanoids. *Anal. Biochem.* 184:1–10.

6. Takahashi, K., J. Capdevila, J. R. Falck, H. R. Jacobson, and K. F. Badr. 1990. Cytochrome P-450 arachidonate in the rat kidney: characterization and hemodynamics responses. *Am. J. Physiol.* 258 (*Renal and Electrolyte Physiol. 27*):F781–F789.

7. Maddox, D. A., D. C. Prince, and F. C. Rector, Jr. 1977. Effect of surgery on plasma volume and salt and water excretion in rat. *Am. J. Physiol.* 233 (*Renal Fluid Electrolyte Physiol. 6*):F600–F606.

8. Führ, J., J. Kazmaczyk, and C. D. Krüttgen. 1955. Eine einfache colorimetrische Methode zur Inulinbestimmung für Nieren-Clearanceuntersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin. Wochenschr.* 33:729–730.

9. Smith, H. W., N. Finkelstein, and L. Aliminosa. 1945. The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.* 24:388–391.

10. Ogletree, M. L., D. N. Harris, R. Greenberg, M. F. Haslanger, and M. Nakane. 1985. Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist. *J. Pharmacol. Exp. Ther.* 234:435–441.

11. Stahl, G. L., H. Darius, and A. M. Lefer. 1986. Antagonism of thromboxane actions in the isolated perfused rat heart. *Life Sci.* 38:2037–2041.

12. Badr, K. F., D. K. DeBoer, K. Takahashi, R. C. Harris, A. Fogo, and H. R. Jacobson. 1989. Glomerular responses to platelet activating factor in the rat: role of thromboxane A_2. *Am. J. Physiol.* 256 (*Renal Fluid Electrolyte Physiol.* 25) F35–F43.

13. Deen, W. M., J. L. Troy, C. R. Robertson, and B. M. Brenner. 1973. Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J. Clin. Invest.* 52:1500–1508.

14. Viets, J. W., W. M. Deen, J. L. Troy, and B. M. Brenner. 1978. Determination of serum protein concentration in nanoliter blood samples using fluorescamine or o-phthaldehyde. *Anal. Biochem.* 88:513–521.

15. Takahashi, K., G. F. Schreiner, K. Yamashita, B. W. Christman, I. Blair, and K. F. Badr. 1990. Predominant functional roles for thromboxane A_2 and prostaglandin E_2 during late nephrotoxic serum glomerulonephritis in the rat. *J. Clin. Invest.* 85:1974–1982.

16. Ross, R. 1971. The smooth mustle cell. II. Growth of smooth mustle in culture and formation of elastic fiber. *J. Cell Biol.* 50:172–186.

17. Hanasaki, K., K. Nakano, H. Kasai, H. Arita, K. Ohtani, and M. Doteuchi. 1988. Specific receptors for thromboxane A_2 in cultured vascular smooth mustle cells of rat aorta. *Biochem. Biophys. Res. Commun.* 150:1170–1175.

18. Hanasaki, K., K. Nakano, H. Kasai, and H. Arita. 1989. Biochemical characterization and comparison of rat thromboxane A_2/prostaglandin H_2 receptors in platelets and cultured aortic smooth mustle cells. *Biochem. Pharmacol.* 38:2967–2976.

19. Badr, K. F., B. M. Brenner, and I. Ichikawa. 1987. Effects of leukotriene D_4 on glomerular dynamics in the rat. *Am. J. Physiol.* 253 (*Renal Fluid Electrolyte Physiol. 22*):F239–F243.

20. Harris, R. C., K. A. Munger, K. F. Badr, and K. Takahashi. 1990. Mediation of renal vascular effects of epidermal growth factor by arachidonate metabolites. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 4:1654–1660.

21. Stier, C. T., Jr., L. Jackson Roberts, II, and P. Y-K. Wong. 1987. Renal response to 9α, 11β-prostagalandin F_2 in the rat. *J. Pharmacol. Exp. Ther.* 217:487–491.

22. Mené, P., and M. J. Dunn. 1988. Eicosanoids and control of mesangial cell contraction. *Circ. Res.* 62:916–925.

23. Badr, K. F., V. E. Kelley, H. G. Renneke, and B. M. Brenner. 1986. Roles for thromboxane A_2 and leukotrienes in endotoxin-induced acute renal failure. *Kidney Int.* 30:474–480.

24. Schlondorff, D. 1987. The glomerular mesangial cell: an expanding role for a specialized pericyte. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 1:272–281.

25. Kaplan, R., H. S. Aynedjian, D. Schlondorff, and N. Bank. 1990. Renal vasoconstriction caused by short-term cholesterol feeding is corrected by thromboxane antagonist or probucol. *J. Clin. Invest.* 86:1707–1714.