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Research Article

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Predominant Functional Roles for Thromboxane A₂ and Prostaglandin E₂ during Late Nephrotoxic Serum Glomerulonephritis in the Rat

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Abstract

While much is known regarding acute nephrotoxic serum (NTS)-induced glomerular injury, the glomerular dynamics and pathophysiologic mediators of the more relevant chronic autologous phase remain poorly defined. Studies were performed in rats 14 d after injection of rabbit serum ($n = 6$), NTS in the absence ($n = 6$), or presence, of a cyclooxygenase inhibitor, ibuprofen ($n = 6$) or a thromboxane A₂ (TxA₂) receptor antagonist, L-670,596 ($n = 5$). A mesangial macrophage/monocyte infiltrate was noted with equal intensity in all NTS-treated rats. Glomerular generation rates of prostaglandin (PG) E₂, PGF₂ₐ, and TxA₂ in nephritic kidneys were dramatically increased as compared to controls. 2 wk after NTS, there was an increase in glomerular plasma flow rate (SNPF), attainment of filtration pressure disequilibrium, and augmentation of net transcapillary hydraulic pressure difference (ΔP). Glomerular filtration rate (GFR), however, was reduced, due to a marked fall in the glomerular capillary ultrafiltration coefficient (K_f). Cyclooxygenase inhibition resulted in normalization of glomerular eicosanoid generation rates, amelioration of proteinuria, afferent vasoconstriction, and normalization of SNPF, ΔP, K_f, and GFR. Selective antagonism of TxA₂ also led to preservation of K_f, but was without effect on SNPF, thereby leading to elevated values for GFR. Thus, in contrast to the pathophysiologic role of arachidonate-lipoxygenase products in the early heterologous phase, PG-mediated vasodilatation and TxA₂-induced reductions in K_f and GFR underlie glomerular functional changes during autologous mesangio-proliferative glomerulonephritis. (*J. Clin. Invest.* 1990. 85:1974–1982.) autologous phase • glomerular hemodynamics • glomerulonephritis • prostaglandin E₂ • thromboxane

Introduction

Human glomerulonephritis is often associated with moderate to severe reductions in the glomerular filtration rate (GFR) despite relative preservation of renal plasma flow (RPF)[1] rate (1, 2). In previous studies, we (3) and others (4–17) have used a rat model of glomerulonephritis, induced by the administration of rabbit anti–rat glomerular basement membrane (GBM) antibody (nephrotoxic serum [NTS]), to define the mechanisms underlying the reductions in GFR and the alterations in renal vascular tone which characterize glomerular inflammatory injury. Two phases of this disease model are recognized: an early (heterologous) phase, occurring 2–24 h after antibody binding to GBM with subsequent complement activation, neutrophil infiltration, and falls in GFR and RPF; and a more chronic (autologous) phase, observed by 7–14 d (19), characterized by proteinuria, a macrophage/monocyte cellular infiltrate, and proliferation of resident glomerular mesangial cells (18–20).

Micropuncture studies in the past have focused on the heterologous phase of injury (3–5, 7, 9) or have used the model of "accelerated NTS nephritis", in which rats are preimmunized with rabbit IgG 2–3 d before NTS administration (20), to evaluate the chronic phase (13–15). Physiologic and biochemical measurements obtained during accelerated NTS nephritis, however, are conflicting and difficult to interpret (13–15) due to the marked heterogeneity of glomerular lesions (18). The histopathologic and functional characteristics of the autologous phase of nonaccelerated NTS nephritis are relatively homogeneous (18–20) and constitute a more faithful representation of their respective counterparts in mild to moderate forms of human glomerulonephritis (1, 2). Little is known, however, regarding the mediators of injury, and no data is available on glomerular dynamics, in this more relevant phase of the disease.

The present studies examine the role of endogenously generated bioactive lipids in mediating glomerular dysfunction during autologous NTS-induced nephritis. We used micropuncture techniques in the presence and absence of cyclooxygenase (CO) inhibition or thromboxane A₂(TxA₂)/prostaglandin (PG) endoperoxide receptor blockade, in combination with measurements of endogenous glomerular eicosanoid generation rates. In addition to defining the glomerular generation rates and target sites of action of PGs, TxA₂, and platelet activating factor (PAF) in this model of injury, our findings may provide insight into the mechanisms underlying the improvement in glomerular function observed after administration of TxA₂ antagonists to patients with active glomerulonephritis (21).

Methods

Preparation of anti-GBM serum

NTS was produced in rabbits by repeated immunization with basement membrane–rich sediment of rat cortex, as previously described (3). The dosage of NTS used in final experiments was determined by examining the volume of serum required to induce moderate proteinuria (20–40 mg protein/day) in the first 24 h after intravenous injection. All rats received a standardized dose of 0.2 ml.

1. *Abbreviations used in this paper:* AP, arterial pressure; CO, cyclooxygenase; FF, filtration fraction; NTS, nephrotoxic serum; PAF, platelet activating factor; RPF, renal plasma flow; SNFF, single nephron filtration fraction; SNPF, single nephron plasma flow; UP, urinary protein excretion.

Induction of NTS and experimental protocols

Adult male Munich-Wistar rats weighing 220–270 g were maintained in metabolic cages on a standard rat diet and allowed free access to water and food. After collection of urine for 24 h for the measurement of base-line protein excretion, 0.2 ml of NTS or 0.2 ml of nonimmune rabbit serum was administered intravenously and a 24-h urine collection was performed daily over the subsequent 14 d. Micropuncture studies, histologic examinations, glomerular isolation and incubation, and preparation of glomerular supernatants and tissue extracts for bioanalytic assays were performed in the same rats 14 d after the administration of NTS. Rats were divided into four experimental groups as follows.

Group I (n = 6). In this group of rats, 0.2 ml of nonimmune rabbit serum, the vehicle for NTS, was administered intravenously on day zero and the above studies and measurements performed on day 14.

Group II (n = 6). In these animals, 0.2 ml of NTS was administered intravenously on day zero and the above studies and measurements performed on day 14.

Group III (n = 6). These animals received NTS as in group II, but were also treated with ibuprofen (The Upjohn Co., Kalamazoo, MI) (20 mg/kg) intraperitoneally on days 11–14 and the above studies and measurements performed on day 14.

Group IV (n = 5). In these rats, NTS was administered as in group II, but the animals were additionally treated with a novel orally acting potent and selective TxA$_2$/PG endoperoxide receptor antagonist, L-670,596 (22) (Kindly provided by Dr. A. Ford-Hutchinson, Merck-Frosst Canada Inc., Pointe Claire-Dorval, Canada). This compound has been shown to inhibit renal vasoconstrictor responses to the TxA$_2$/PG endoperoxide mimetic, U-44069, with an ED50 value of 0.02 mg/kg, i.v. (22). In the present studies, L-670,596 was administered orally in a dose of 10 mg/kg every 8 h on the day before the experiment and just before the micropuncture study. This frequency of administration is based on the determined half-life for this compound in rat plasma.

Micropuncture studies

Rats were prepared for micropuncture according to protocols described previously (3). In brief, after Inactin anesthesia (100 mg/kg, i.p.; Byk Gulden Lomberg Chemische Fabrik GmbH, Konstanz, Federal Republic of Germany), the left femoral artery was catheterized with PE 50 tubing which was used to monitor mean systemic arterial pressure (AP) by means of a pressure transducer (P23Db; Statham Instruments) connected to a direct writing recorder (Gould Instruments Inc., Cleveland, OH) and for sampling of blood. After tracheostomy, polyethylene catheters were inserted into both jugular veins for infusion of plasma and a solution of ^{3}H-inulin (300 μCi per experimental period in 0.9% NaCl) and para-aminohippurate (0.32 mg/min) at 1.2 ml/h. The left kidney was exposed on a Lucite holder. The kidney surface was illuminated with a fiberoptic light source and bathed with isotonic NaCl. Homologous rat plasma was administered intravenously at a rate of 10 ml/kg per hour for 45 min followed by a reduction in infusion rate to 1.5 ml/kg per hour for the remainder of the experiment. This protocol of plasma administration has been shown previously to adequately replace surgically induced plasma losses, thus maintaining euvolemia (23). In all experiments micropuncture measurements were started 45 min after the onset of plasma infusion and carried out as follows. Exactly timed (1–2 min) samples of fluid were collected from surface proximal convolutions of each of three to five nephrons for determination of flow rate and inulin concentration and calculation of tubule fluid-to-plasma inulin concentration ratio and single nephron glomerular filtration rate (SNGFR). Coincident with these tubule fluid collection, two to three samples of femoral arterial blood were obtained in each period for determination of systemic arterial hematocrit (Hct) and plasma concentration of total protein and inulin. In addition, two or three samples of urine from the experimental kidney were collected for the determination of flow rate and inulin concentration, and for the calculation of whole kidney GFR. For these urine collections, indwelling ureteral catheters were used (PE 10).

Time-averaged hydraulic pressures were measured in surface glomerular capillaries (P$_{GC}$), proximal tubules (P$_T$), and surface efferent arterioles (P$_E$) using a continuous recording, servo-null micropipette transducer system (model 5; Instrumentation for Physiology & Medicine, In., San Diego, CA). Micropipettes with outer tip diameters of 2–3 μm and containing 2.0 M NaCl were used. Hydraulic output from the servo-nulling system was coupled electronically to a second channel of the Gould recorder by means of a pressure transducer.

Analytical

Colloid osmotic pressures of plasma entering and leaving glomerular capillaries were estimated from values for protein concentrations (C) in femoral arterial (C$_A$) and surface efferent arteriolar (C$_E$) blood plasmas. Colloid osmotic pressure (π) was calculated according to previously derived equations (24). Values for C$_A$, and thus π$_A$, for femoral arterial plasma are taken as representative for values for C and π for the afferent end of the glomerular capillary network. These estimates of pre- and postglomerular protein concentration permit calculation of single nephron filtration fraction (SNFF), and glomerular capillary ultrafiltration coefficient (K$_f$), as well as resistance of single afferent (R$_A$) and efferent (R$_E$) arterioles, and initial glomerular capillary plasma flow (SNPF), using equations described in detail elsewhere (24).

The concentrations of inulin in tubule fluid, plasma and urine were determined by measuring the radioactivity of ^{3}H-inulin in a scintillation counter (Beckman Instruments Inc., Palo Alto, CA). Calculation of values for GFR and SNGFR were performed using conventional formulae. The concentration of para-aminohippurate in urine and plasma was determined according to the method of Smith et al. (25). Protein concentration in efferent arteriolar and femoral arterial blood plasmas were determined using a fluorometric method developed by Viets et al. (26).

Immediately after termination of micropuncture measurements, kidneys were perfused in situ with a physiologic buffer (buffer A, see below) and glomeruli were isolated by a modified differential sieving technique for analysis of prostanoid generation (groups I–IV) and platelet activating factor (PAF) content (groups I–III), as detailed below.

In selected animals in each group, kidneys were perfused and then prepared for histologic examination. For evaluation of glomerular pathology, kidneys were fixed in Karnovsky's fixative. 4–6-μm paraffin sections were stained with hematoxylin and eosin (H+E).

Immunohistochemical labeling of glomeruli

Frozen sections (6 μm) of kidneys harvested from (1) control animals, (2) nephritic animals (day 14), (3) and nephritic animals (day 14) treated with ibuprofen and the TxA2 receptor antagonist, were fixed in chilled (4°C) acetone for 10 min and air dried. Endogenous biotin activity was inhibited by sequential 30-min incubation in avidin D and biotin blocking solutions (Vector Laboratories Inc., Burlingham, CA). The sections were then placed in a blocking solution of 10% horse serum (Vector Laboratories Inc.) and decomplemented by heating for 15 min. The sections were labeled for infiltrating macrophages with the monoclonal mouse antibody ED1 (Accurate Chemical & Scientific Corp., Westbury, NY), specific for a cytoplasmic antigen of rat monocytes and macrophages (27), at 50 μg/ml for 1 h at room temperature. After washing in 0.01 M PBS, the sections were placed in biotinylated horse anti–mouse IgG (Vector Laboratories Inc.), adsorbed against rat IgG, and diluted 1:50 in PBS for 30 min at room temperature. After washing, the slides were sequentially exposed to streptavidin-peroxidase followed by aminoethyl carbazole and hydrogen peroxide, as supplied by Zymed Laboratories (San Francisco, CA). All glomeruli on a section were scored for positive labeled cells and the results calculated on a per glomerular basis (n = 15–25 glomeruli per group).

Glomerular isolation, eicosanoid generation assay, and PAF analysis

Glomeruli were isolated by a modification of the differential sieving technique, aimed at optimizing glomerular viability (28). In brief, the

removed kidneys were immediately placed in a physiologic buffer (buffer A) consisting of 105 mM NaCl, 24 mM NaHCO₃, 5 mM KCl, 2 mM Na₂HPO₄, 1 mM MgSO₄, 1.5 mM CaCl₂, 5 mM glucose, 1 mM L-alanine, 10 mM Hepes, and 0.2% BSA, pH 7.4, which was prebubbled with 95% O_2/5% CO_2 for 30 min. The cortical tissue was then minced at 4°C and transferred to another beaker containing a solution of 0.3 mg/ml collagenase Sigma, Type I, (Sigma Chemical Co., St. Louis, MO) in buffer A, and bubbled in this solution with 95% O_2/5% CO_2 for 45 min at 37°C. Differential sieving was then carried out by passing cortical tissue sequentially through 150- and 75-μm sieves, with warming and gentle centrifugation. The glomerular preparation that was obtained was > 95% pure. The isolated glomeruli were then incubated in buffer A maintained at 37°C and 95% O_2/5% CO_2 for 1 h. The preparation was then centrifuged and divided into the supernatant and the glomerular pellet. The supernatant was frozen at −70°C for eicosanoid generation assays at a later time, and the glomeruli were extracted for measurement of PAF content as described below. The protein content of the pelleted glomeruli was assayed by a colorimetric method.

The amounts of PGE₂, PGF₂ₐ, and TxB₂, the stable metabolite of TxA₂, generated by normal and nephritic glomeruli were determined by radioimmunoassay (New England Nuclear, Boston, MA). This was performed after separation of prostanoid-containing fractions of the glomerular supernatants by subjecting the acidified samples to Sep-Pak C-18 prewashed with water and methanol, followed by elutions with water, ethanol-water (15/85, [vol/vol]), petroleum ether, and finally methyl formate. The final fraction was evaporated with N₂ and reconstituted in phosphate-buffered saline (29).

PAF analysis

Glomerular PAF content was determined by gas chromatography/mass spectrometry using a recently described method applied to the determination of salivary PAF concentrations by Christman and Blair (30). Pelleted glomeruli were resuspended in methanol and sonicated for 1 min. The mixture was "spiked" with 10 ng of synthetically prepared deuterated PAF. Lipids were extracted by the method of Bligh and Dyer (31) with the removal of the protein precipitate (saved for later determination of protein content) during the organic monophase. The organic phase was applied to a preconditioned silica minicolumn washed sequentially with 1% acetic acid in chloroform and chloroform/methanol (1:1, [vol/vol]), and PAF eluted with CHCl₃/CH₃OH/H₂O (1.25:2.50:1.0, [vol/vol/vol]). The Bligh-Dyer organic phase was removed and dried under N₂.

The phosphocholine chain was removed by incubation with 6 U of phospholipase C (Sigma Chemical Co.) in a mixture on 0.1 N Tris buffer with 2 mM CaCl₂ for 2 h at 37°C in a shaking water bath. The resultant alkyl acetyl glycerol was extracted with ethyl acetate, concentrated under N₂, and purified by thin layer chromatography (TLC) using hexane/ethyl acetate (1:1, [vol/vol]) as the mobile phase. After localization with a tritiated standard, appropriate silica segments were removed and eluted with 1.4 ml of the mobile phase. Samples were dried under N₂, dissolved in toluene, and derivatized with pentafluorobenzoyl chloride (Aldrich Chemical Co., Milwaukee, WI) using a catalytic amount of pyridine. Samples were dried, transferred to TLC plates, and developed to 15 cm in hexane/ethyl acetate (9:1, [vol/vol]). The derivatized compounds were again localized by comparison to a tritiated standard, eluted from silica, and dissolved in decane. Gas chromatography/mass spectrometry was then performed as described in (30).

Statistical

Intergroup comparisons were performed by ANOVA. Differences were considered significant at a P value ≤ 0.05. All values are reported as mean±SEM.

Results

Morphologic examination. Light microscopic examination in rabbit serum-treated animals (group I) revealed normal appearing glomeruli (Fig. 1 *A*). In animals treated with NTS (group II) light microscopic examination of the 14-d lesion revealed a mesangioproliferative form of glomerulonephritis with enlarged glomeruli, mesangial expansion, and mesangial hypercellularity, with preservation of the glomerular capillary thickness (Fig. 1 *B*). These changes were present in all glomeruli examined and the intensity of the mesangial infiltrate was homogeneous. Cyclooxygenase inhibition in NTS-treated rats with ibuprofen (group III) and L-670,596 was not associated with amelioration of the mesangial cellular infiltrate (Fig. 1 *C*). Indeed, there appeared to be evidence of exacerbation of the lesion, with many glomeruli evincing diffusely proliferative histology. Labeling of glomerular sections for the macrophage cytoplasmic marker ED-1, revealed that the predominant cell type in the mesangial cellular infiltrate was composed of a macrophage/monocyte population (Fig. 1 *D*). Glomerular macrophage labeling in control kidneys equalled 0.3±0.1 macrophages/glomerulus. After 14 d of nephritis, there was marked increase to 7.8±0.5 macrophages/glomerulus. Nephritic animals receiving ibuprofen exhibited no diminution in the monocytic invasion of the glomerulus (9.2±0.7 macrophages/glomerulus), nor did those receiving L-670,596 (11.9±0.5 macrophages/glomerulus).

Micropuncture studies. Mean values for Hct, AP, GFR, RPF, FF, and urinary protein excretion in the four groups of animals are summarized in Table I. No significant differences in Hct were observed among the four groups. The autologous phase of anti–GBM-induced glomerulonephritis (group II) was not associated with significant changes in AP, as compared to normal controls (112±5 vs. 114±5 mmHg, NS). Treatment with ibuprofen in group III rats, however, was associated with an elevated value of AP as compared to both control animals (125±1 vs. 114±5 mmHg in group I; $P < 0.01$), as well as nonibuprofen-treated glomerulonephritic rats in group II ($P < 0.01$). No elevation in AP was observed in group IV animals which were treated with the TxA₂ receptor antagonist, L-670,596, when compared to control rats (110±5 mmHg; NS vs. group I). AP in group IV animals, however, was significantly less than that observed in ibuprofen-treated group III rats ($P < 0.01$ vs. group III) (See Table I).

GFR, as measured by inulin clearance, was mildly, but significantly, depressed in group II animals as compared to group I controls (1.00±0.07 vs. 1.18±0.03 ml/min; $P < 0.05$). Treatment with ibuprofen in group III was associated with full restoration of GFR (1.16±0.07; $P < 0.05$ vs. group II and NS vs. group I) despite the continued presence of significant inflammatory reaction on histologic examination (Fig. 1). Of interest, the values for GFR in L-670,596-pretreated animals in group IV were significantly higher than those obtained in normal, nonnephritic animals (group I) (1.30±0.06 ml/min; $P < 0.05$ vs. group I and $P < 0.01$ vs. group II). RPF, as calculated from para-aminohippurate clearance, was significantly greater in group II animals than that of group I controls (5.66±0.42 vs. 4.63±0.26 ml/min; $P < 0.05$). However, treatment with ibuprofen in glomerulonephritic group III rats was associated with an appreciable reduction in RPF to values below those observed in NTS-treated group II animals (4.32±0.23 ml/min; $P < 0.01$) and significantly less than those in vehicle-treated normal controls (group I; $P < 0.05$). The renal vasodilatation which characterized the kidneys of glomerulonephritic animals in group II was not affected by TxA₂ receptor antagonism in group IV, in which the mean value for RPF was 5.74±0.33 ml/min (significantly higher than that of
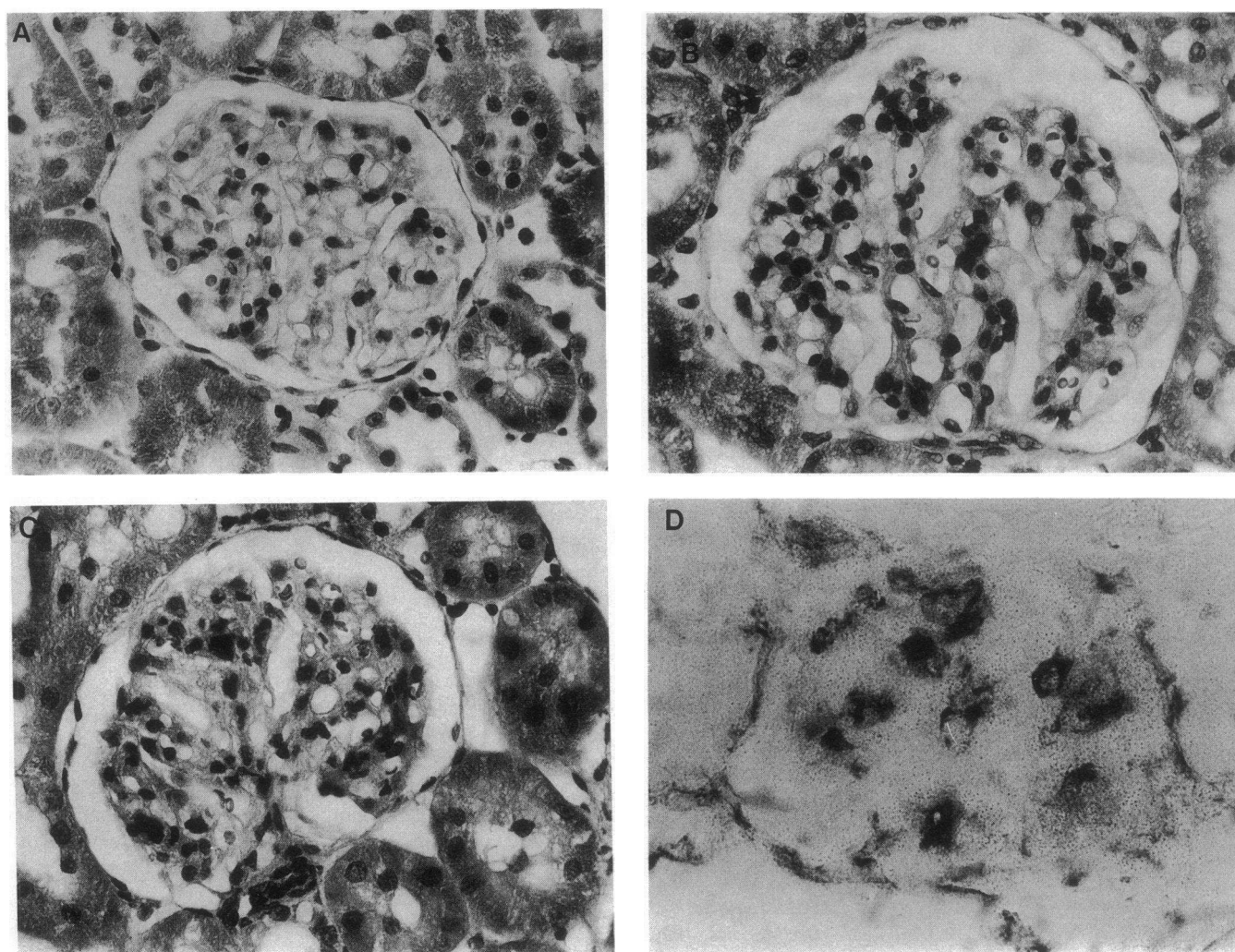
Figure 1. (*A*) Normal rat glomerulus (group I), (H + E). (*B*) Glomerulus 14 d after NTS (group II), (H+E). (*C*) Glomerulus 14 d after NTS with ibuprofen treatment (group III), (H+E). (*D*) Glomerulus from a group II rat labeled for macrophages with ED1. The antibody detects a cytoplasmic antigen (×240).

groups I and III rats, see Table I). Thus, the mean values for filtration fraction (FF) in groups II and IV animals were 0.18 ± 0.01 and 0.21 ± 0.02, respectively, values significantly lower than those of group I (0.25 ± 0.01; $P < 0.01$ vs. group II, $P < 0.05$ vs. group IV) and group III (0.28 ± 0.01; $P < 0.01$ vs. group II and IV).

In parallel with these changes in whole-kidney GFR, RPF, and FF, single nephron measurements revealed a fall in SNGFR and SNFF, and an increase in SNPF in group II rats as compared to group I controls (33.5 ± 2.4 vs. 38.9 ± 1.1 nl/min; $P < 0.05$, 0.19 ± 0.01 vs. 0.26 ± 0.01; $P < 0.01$, and 149 ± 7 vs. 178 ± 8; $P < 0.01$, respectively). Similarly, administration of

Table I. Values for Hematocrit, Mean Arterial Pressure, GFR, RPF, FF, and UP in Groups I–IV

Group	Hct	MAP	GFR	RPF	FF	UP		
						Day 0	Day 11	Day 14
	% vol	*mmHg*	*ml/min*	*ml/min*			*mg/24 h*	
I	48.5±0.9	114±5	1.18±0.03	4.63±0.26	0.25±0.01	7.6±0.8	8.4±0.3	8.5±0.3
II	47.3±0.9	112±5	1.00±0.07*	5.66±0.42*	0.18±0.01*	6.1±0.9	18.6±2.8	17.2±2.7*
III	47.5±0.9	125±5*	1.16±0.07	4.32±0.23*†	0.28±0.01	4.0±0.6	14.1±1.3	9.7±1.3§
IV	46.2±0.9	110±5	1.30±0.06*†	5.74±0.33*	0.21±0.02*	9.9±1.2	20.4±2.6	19.4±1.6*

All values are given as mean ± SEM. Hct, hematocrit; MAP, mean arterial pressure. *, $P < 0.05$ vs. group I; †, $P < 0.05$ vs. group II; §, $P < 0.05$ vs. day 11.

the CO inhibitor in group III was associated with preservation of SNGFR (38.5 ± 2.4 nl/min; $P < 0.05$ vs. group II and NS vs. group I) and a decrement in SNPF (136 ± 10 nl/min, $P < 0.005$ vs. group II), while pretreatment with the TxA_2 receptor antagonist in group IV was associated with augmentation of SNGFR (43.5 ± 1.9 nl/min; $P < 0.01$ vs. group II; $P < 0.05$ vs. group I), and persistent glomerular hyperperfusion (SNPF = 170 ± 12 nl/min, a value significantly higher than that of group I and group III animals [$P < 0.05$], but not different from that of group II rats) (Fig. 2).

Hydraulic pressure measurements in glomerular capillaries, proximal tubules, and efferent arterioles revealed a mild, but significant, elevation in the mean value for P_{GC} in group II rats as compared to group I controls (50 ± 1 vs. 47 ± 2 mmHg, $P < 0.05$). Mean P_{GC} values in groups III and IV were intermediate between those obtained in groups I and II, and not significantly different from either (48 ± 1 and 49 ± 3 mmHg, respectively). The mean values for proximal tubule pressure, P_T, was significantly depressed in group II animals, as compared to the other three groups of rats (9 ± 1.0 mmHg in group II, $P > 0.01$ vs. 13 ± 1 mmHg in group I, 12 ± 1 mmHg in group III, and 13 ± 1 mmHg in group IV, respectively). The combined increase in P_{GC} and decrease in P_T observed in group II resulted in appreciably greater values for the mean transcapillary hydraulic pressure difference, ΔP, in this group of animals (42 ± 1 mmHg), as contrasted to groups I (35 ± 1 mmHg, $P < 0.0005$ vs. group II), III (36 ± 1 mmHg; $P < 0.01$ vs. group II), and IV (37 ± 2 mmHg; $P < 0.05$ vs. group II) (Fig. 3). Pressures in surface efferent arterioles, P_E, were not significantly different among the four groups: group I, 14 ± 1 mmHg; group II, 14 ± 1 mmHg; group III, 13 ± 1 mmHg; group IV, 13 ± 1 mmHg. Mean values for postglomerular oncotic pressure (π_E) in groups I, II, III, and IV were 26 ± 1, 24 ± 1, 30 ± 2, and 28 ± 1 mmHg, respectively. Of interest, values for the ratio of $\Delta P/\pi_E$ for these groups were 1.3 ± 0.1, 1.8 ± 0.1, 1.2 ± 0.1, and 1.4 ± 0.2,

Figure 3. Mean values for mean net transcapillary hydraulic pressure difference (ΔP) for groups I–IV. See the legend to Fig. 2 for explanation of symbols. Note the elevated values for ΔP in GN animals and its abrogation by IBU and L67.

respectively. Only in group II animals was this ratio sufficiently different from unity and from that which obtained in control (or treated) rats ($P < 0.025$ vs. group I and II and $P < 0.05$ vs. group IV) to define the presence of filtration pressure disequilibrium. Calculation of preglomerular and postglomerular arteriolar resistances, R_A and R_E, revealed that group II animals were characterized by a significant selective reduction in R_A as compared to group I controls (R_A: 1.52 ± 0.11 vs. 1.87 ± 0.12 10^{10}dyn$\cdot$s$\cdot$cm^{-5}; $P < 0.05$; R_E: 1.02 ± 0.05 vs. 1.13 ± 0.11 10^{10}dyn$\cdot$s$\cdot$cm^{-5}; NS). Treatment with ibuprofen in group III and L-670596 in group IV animals modified these changes in pre- and postglomerular resistances dramatically. Preglomerular arteriolar resistance in group III was increased significantly as compared to groups I and II (2.39 ± 0.25 10^{10}dyn$\cdot$s$\cdot$cm^{-5}; $P < 0.05$ vs. group I and $P < 0.01$ vs. group II). Postglomerular resistance was not statistically different from group I, but significantly greater than that of group II (1.02 ± 0.05 10^{10}dyn$\cdot$s$\cdot$cm^{-5}; NS vs. group I and $P < 0.05$ vs. group II). In group IV, however, R_A remained near normal at 1.67 ± 0.12 10^{10}dyn$\cdot$s$\cdot$cm^{-5}, and the mean value for R_E was also similar to that measured in control rats (1.10 ± 0.06 10^{10}dyn$\cdot$s$\cdot$cm^{-5}), but significantly lower than that of group III ($P > 0.05$). Changes in arteriolar resistance in the four groups of animals are shown in Fig. 4.

Calculation of the glomerular capillary ultrafiltration coefficient, K_f, in group II showed a dramatic reduction in the mean value for this parameter as compared to controls
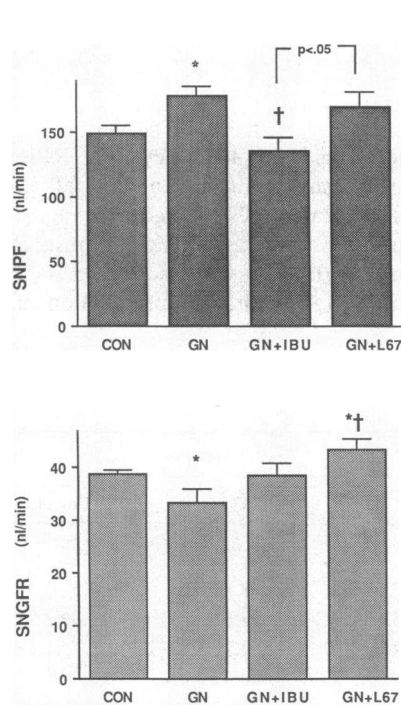
Figure 2. Mean values for single nephron plasma flow rates (SNPF, top) and glomerular filtration rates (SNGFR, bottom) for group I (CON, control), group II (GN, glomerulonephritic), group III (GN + IBU, GN in the presence of ibuprofen), and group IV (GN + L67, GN in the presence of the TxA_2 receptor antagonist, L-670,596). *, statistically different from CON; †, statistically different from GN. See text for P values. Note the increased values for SNPF in non–CO-inhibited animals (GN and GN + L67) and its marked depression in the presence of IBU. Note the restoration of SNGFR in GN + IBU rates, and its supranormal values in GN + L67 animals.
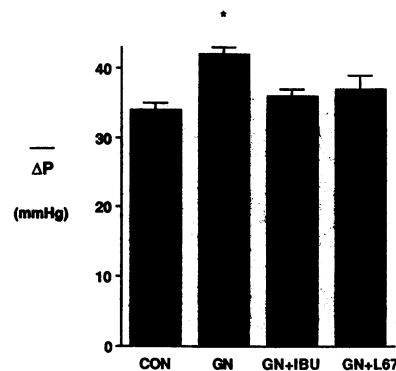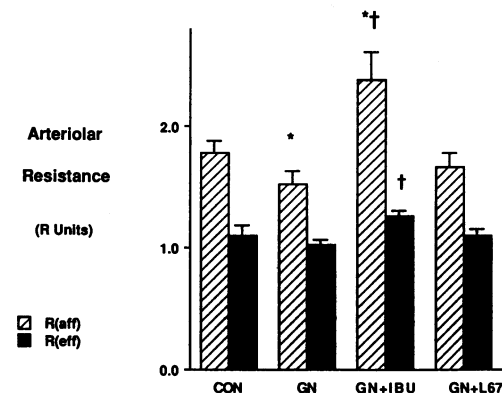
Figure 4. Mean values for preglomerular (striped bars) and postglomerular (solid bars) arteriolar resistances in groups I–IV. See the legend to Fig. 2 for explanation of symbols. Note the lower values for preglomerular resistance in glomerulonephritic animals and the increases in both pre- and postglomerular resistances upon treatment with IBU. See text for further discussion.
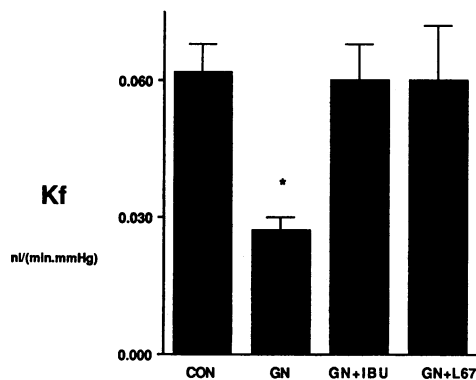
Figure 5. Mean values for the glomerular capillary ultrafiltration coefficient, K_f, in groups I–IV. See the legend to Fig. 2 for explanation of symbols. Note the reduction in this parameter in GN animals and its restoration in both GN + IBU–treated and GN + L67–treated rats.

$(0.027\pm0.003$ vs. 0.064 ± 0.005 nl/[s·mmHg] in group I; $P < 0.01$). However, this marked fall in K_f was totally prevented by treatment with a CO inhibitor in group III $(0.060\pm0.008$ nl/[s·mmHg]; $P < 0.01$ vs. group II), or by the presence of the TxA_2 receptor antagonist in group IV $(0.060\pm0.012$ nl/[s·mmHg]; $P < 0.01$ vs. group II). Fig. 5 shows the mean values for K_f in the three groups of animals.

Lipid generation rates. Glomeruli freshly isolated from NTS-treated group II rats were characterized by dramatically and significantly stimulated endogenous generation rates of PGE_2, $PGF_{2\alpha}$, and TxB_2 by as much as 150, 78, and 95%, respectively, as compared to those obtained from normal controls (group I). Pretreatment with ibuprofen in group III totally abrogated the enhanced glomerular eicosanoid biosynthetic capacity, despite the continued presence of an inflammatory reaction in these glomeruli (See Figs. 1 and 6). In rats treated with L-670,596, antagonism of TxA_2 receptors was not associated with modification of the enhanced glomerular TxA_2 synthesis, which increased significantly by 110% over control. Analysis of the extract from the incubated glomeruli indicated the presence of $C_{16:0}$ PAF in all groups. Measurement of ion ratios in conjunction with a standard curve showed no significant differences in the level of $C_{16:0}$ PAF in glomeruli obtained from rats in groups I, II, and III. The values were 517 ± 51 (range: 386–632) in controls (group I), 416 ± 43 (325–565) in NTS-treated rats (group II), and 461 ± 53 (370–665) pg/mg protein in NTS-treated animals which received ibuprofen (group III). These results are shown in Fig. 7.

Table I summarizes the changes in total urinary protein excretion (*UP*) in all groups of animals. In group II rats, UP increased from a baseline value of 6.1 ± 0.9 to 18.6 ± 2.8 mg/day
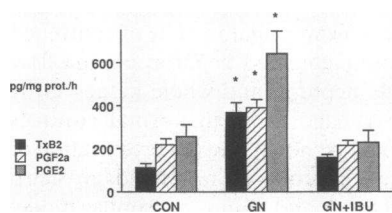

Figure 6. Mean values for glomerular generation rates of TxB_2 (*solid bars*), $PGF_{2\alpha}$ (*striped bars*), and PGE_2 (*stippled bars*) in groups I–III. See the legend to Fig. 2 for explanation of symbols. Note the stimulation of eicosanoid synthesis in GN animals and its restoration to control values by ibuprofen. Note that these are endogenous glomerular generation rates in the absence of excess substrate or in vitro stimulation.
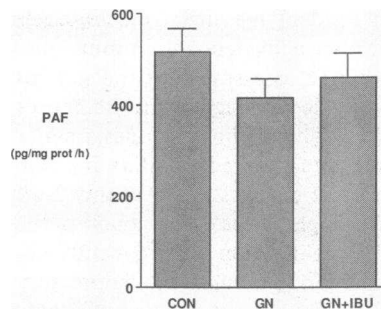

Figure 7. Glomerular PAF generation rates in pg/mg protein per minute in glomeruli from groups I–III, as measured by GC/MS. See the legend to Fig. 2 for explanation of symbols. No significant changes were noted among the three groups.

$(P < 0.01)$ 1 d post-NTS injection and remained increased for the remaining 14 d of observation. Treatment with ibuprofen for 3 d in group III rats suppressed the increased protein excretion which characterized these rats at day 10 from 14.1 ± 1.3 to 9.7 ± 1.3 mg/day $(P < 0.01)$, a value which was not significantly different from that observed in group I control rats. This suppression of elevated UP was not observed in group IV animals $(20.4\pm2.6$ and 19.4 ± 1.6 mg/day, before and after the treatment with L-670596, respectively).

Discussion

Evidence from numerous investigators (3, 8, 10, 11, 16), has established a role for biologically active cyclooxygenase (CO) and lipoxygenase-derived products of arachidonic acid metabolism in mediating the impairments in glomerular perfusion and filtration functions which attend heterologous NTS-induced injury. Little is known, however, regarding the pattern of glomerular dynamics, or the mechanisms involved in mediating functional impairment, during the autologous phase. Studies by Maddox et al. (6) were performed between days 2 and 16 after administration of antibody, a time frame during which a wide variety of histologic and functional changes occur (19). Other studies by Sakai et al. (13), Allison et al. (14), and Kaizu et al. (15) were performed using the "accelerated" form of the disease, resulting in a marked increase in severity and heterogeneity of the glomerular morphologic lesions (18, 20). Micropuncture measurements in these studies were also performed over a variable time period of 5–15 d (Sakai et al.) and 10–38 d (Allison et al.) after NTS administration and the roles of endogenous mediators were not investigated. In the one study in which NTS was administered without earlier immunization and in which the role of endogenous eicosanoid generation was investigated (16), no micropuncture data is available, and the physiologic measurements at 14 d after NTS were obtained under hydropenic conditions, which profoundly alter glomerular dynamics during experimental glomerular injury (13) and markedly exaggerate the renal hemodynamic responses to CO inhibition and the physiologic significance of endogenous angiotensin II release (15, 23).

In the present studies, measurements are performed at a single time point after anti–GBM antibody injection (14 d), under euvolemic conditions, and in the setting of the relative homogeneity of the glomerular lesions afforded by the nonaccelerated model of injury. It was our intention to induce a mild form of injury, similar to that seen in the early-to-moderately-advanced forms of human glomerulonephritides. The lesion obtained (Fig. 1) is characterized by a mesangioproliferative histology, consisting of proliferating mesangial cells and infiltrating cells of macrophage/monocyte origin. A number of the tissue macrophages detected by immunostaining in NTS-

treated rats may represent resident mesangial macrophages (32), which are known to become activated after immune-mediated injury (33). Quantitative assessment of the macrophage/monocyte infiltrate revealed no significant differences between groups II, III, and IV. This finding excludes a major role for CO products in mediating leukocyte influx into the glomerulus. While the histologic changes noted at this 2-wk time point may not persist throughout the entire course of the autologous phase, mesangial proliferation is a frequently observed early finding in several forms of human inflammatory glomerular diseases, rendering the present results potentially relevant.

Induction of autologous NTS nephritis in group II rats was not associated with significant alterations in AP or Hct. Of interest, however, CO inhibition in group II animals resulted in values for AP significantly above those obtained in normal controls (group I) or in non-CO inhibited nephritic rats (groups II and IV) (Table I). The increase in AP in group III is likely due to the inhibition of the enhanced renal generation of vasodilator PGs (PGE_2) during the course of chronic glomerulonephritis, resulting in renal vasoconstriction.

Values for GFR in group II NTS-treated rats were moderately, but significantly, depressed as compared to controls (Table I and Fig. 2). In contrast, RPF and single nephron plasma flow rates were higher in glomerulonephritic kidneys than in control animals (Table I and Fig. 2), a finding similar to that observed by Stork and Dunn (16). The relative preservation, or even augmentation, of RPF has also been observed in humans with active glomerulonephritis by Friedman et al. (2) and Meyers et al. (1). The mechanism underlying the increase in renal perfusion is the selective relaxation of preglomerular resistance (R_A) in group II animals as compared to those in group I (Fig. 4). This is likely due to the local release of a vasorelaxant CO product of arachidonic acid, as evidenced by the marked increase in R_A in group III rats in which NTS-treated animals received ibuprofen for the 3 d preceding the experiment (Fig. 4). A role for PGE_2 is suggested by the marked stimulation of its glomerular biosynthesis in group II rats and its effective inhibition in group III (Fig. 6). Of interest, the value for R_A in group III animals exceeded not only that obtained in group II, but also that of normal controls (group I). This suggests the presence of potent non-CO-dependent vasoconstrictor influences in glomerulonephritic rats, whose actions are antagonized by locally generated PGE_2. The predominant afferent action of these putative mediators argues against the involvement of angiotensin II or peptidoleukotrienes, which exert their constrictor actions predominantly on post-glomerular arterioles (34, 35). These findings attest to the complex nature of the neurohumoral systems which mediate the functional derangements during glomerular inflammation.

The augmentation of net mean transcapillary hydraulic pressure difference, ΔP, in group II rats was due to a relatively greater reduction in R_A as compared to R_E (and hence increased P_{GC}), as well as lower values for proximal tubule pressure, P_T, observed in group II rats. The near-normal values of P_{GC} and P_T in groups III and IV was associated with mean ΔP values which are not significantly different from controls (group I) (See Fig. 3).

Despite the higher value of single nephron plasma flow rate (SNPF) and ΔP which characterized animals in group II, GFR was reduced (Table I and Fig. 2) due to the markedly depressed value of the glomerular capillary ultrafiltration coefficient (K_f) (Fig. 5). Low K_f values have been implicated in the reduction

in GFR in the heterologous phase (3–5), as well in studies (6, 13) in which glomerular dynamics were measured between days 2 and 16, and 5 and 15, after administration of antibody. Friedman et al. (2), in their studies on the determinants of glomerular capillary wall function in patients with lupus nephritis, observed a similar profile of glomerular dynamics (assessed by differential solute clearance measurements coupled with mathematical modeling) in which RPF was preserved, but the filtration fraction was low due to a profound reduction in K_f. In view of the relative sparing of the glomerular capillary wall, as assessed by histologic examination, in our nephritic animals (Fig. 1), it is reasonable to assume that the observed reduction in K_f is due to the concerted contractile action of glomerular mesangial cells resulting in a reduction in the capillary surface area available for filtration (3). That this fall in K_f is indeed of a functional nature, is further supported by its abrogation in both groups III and IV, in which the local synthesis, or the receptor occupancy, of the potent vasoconstrictor, TxA_2, is inhibited. (Fig. 5). These findings from glomerular micropuncture, the enhanced glomerular TxA_2 generation rate in NTS-treated rats (Fig. 6), and the histologic finding of inflammatory cells restricted to the mesangial region (Fig. 1), suggest strongly that the intramesangial release of TxA_2 is the principal mechanism underlying the reductions in K_f and GFR in this model of glomerular injury. The functional nature of the fall in K_f is likely a hallmark of the early stages of glomerular injury, when capillary loop and epithelial cell integrity are relatively preserved, as in the present studies. Furthermore, the degree of vasodilatation in group II rats was sufficient to induce filtration pressure disequilibrium in these animals ($\Delta P/\pi_E = 1.8\pm0.1$). This profile of glomerular dynamics renders filtration rates in these animals highly dependent on the values of K_f, thereby providing the theoretical basis for the improvement in GFR upon reversal of the functional reductions in K_f, as seen in groups III and IV. Clearly, this dependence of GFR on K_f would have been unlikely had the inflammatory lesion been associated with renal vasoconstriction, whereby filtration pressure equilibrium prevails. The greater degree of variability among the standard error values for SNGFR in groups II–IV, despite absence of morphological heterogeneity, suggests further that the alterations in glomerular dynamics in nephritic animals are likely dictated by the degree of cellular activation and autacoid release, which are inherently variable, rather than the physical effects of cellular infiltration. The reduction in K_f during more advanced stages of injury, however, is more likely to result from destruction and/or obstruction of capillary loops, rather than to their acute, eicosanoid-mediated, reversible, obliteration by mesangial cell contraction. The capacity of TxA_2 to contract mesangial cells has been demonstrated convincingly by Scharschmidt et al. (36). This TxA_2-mediated fall in K_f is abrogated in group III, with resultant preservation of GFR, and in group IV (Table I and Fig. 2). In the latter, however, and in view of continued afferent vasodilatation and augmented nephron plasma flow rate (Figs. 2 and 4), single nephron and whole kidney GFR attained values which exceeded those in normal controls (Table I and Fig. 2). It should be noted that the preservation of GFR during CO inhibition in group III rats stands in sharp contrast to the findings of Stork and Dunn in a similar model (16) in which no micropuncture measurements are available, but GFR and RPF fell precipitously after administration of CO inhibitors. As noted earlier, however, those studies were performed under hydropenic conditions which are known to

alter glomerular dynamics during NTS-induced injury (13) and under which RPF and GFR are exquisitely sensitive to CO inhibition, due to the elevated endogenous levels of angiotensin II (37).

The generation rates of PGE_2, $PGF_{2\alpha}$, and TxA_2 were measured in the supernatants of intact glomeruli without the addition of exogenous arachidonate or artificial stimuli, such as calcium ionophores. Since intracellularly generated PGs and TxA_2 are rapidly released to the extracellular milieu (38), the measured generation rates likely reflect differences in in vivo intraglomerular eicosanoid release. As seen in Fig. 6, glomerulonephritic injury is associated with dramatically enhanced glomerular generation of both vasodilator (PGE_2) as well as vasoconstrictor (TxA_2 and $PGF_{2\alpha}$) CO products of arachidonic acid. Normal glomeruli, as well as glomerular endothelial, epithelial, and mesangial cells in culture are capable of synthesizing these products under normal resting conditions, as well as in response to various hormonal, physical, or immunologic stimuli (38). The mesangial cellular infiltrate is also a likely source of eicosanoid generation. Recent studies (3, 10, 11) have clearly demonstrated that, in contrast to the marked enhancement of glomerular 5- and 12-lipoxygenase activities seen in the first 2–24 h after NTS administration, no evidence for lipoxygenase product generation can be seen in glomeruli obtained beyond that period of time. In view of the marked alterations in hemodynamics seen at the 14-d time point by us and others upon selective CO inhibition, it thus seems likely that AA metabolism undergoes a shift from lipoxygenase to CO products over the time period during which the cellular infiltrate changes from a neutrophilic to a macrophage/monocytic one. The mechanisms underlying this phenomenon remain to be explored.

The percent increase in glomerular eicosanoid generation in our studies (Fig. 6) is considerably less than the 15-fold increase observed by Stork and Dunn (16) in a similar experimental model. In those studies, however, glomeruli were incubated in the presence of 5 μg/ml ($\sim$ 16 μM) of exogenously added arachidonic acid, a condition which alters markedly the rates of eicosanoid generation (28). In addition, those glomeruli were isolated from kidneys subjected to hydropenic conditions, which are known to stimulate renal prostaglandin synthesis (37, 38), and measurements were performed using a glomerular isolation technique which has since been demonstrated to contain a significant percentage of cells undergoing anoxic injury (28), a condition also known to be associated with increased arachidonic acid release (39).

In view of increasing interest in the glomerular actions and pathophysiologic significance of PAF in models of renal injury, including rabbit NTS nephritis (40), and in light of our recent demonstration that the glomerular actions of PAF were mediated through the secondary glomerular release of TxA_2 (41), we assessed its generation rates in our experimental animals. Gas chromatography/mass spectrometry is the most reliable method currently available to measure PAF generation in biological samples (42). No enhancement of PAF biosynthesis was observed in group II rats, and no modification of its baseline synthetic rates was noted in ibuprofen-treated animals in group III (Fig. 7). It thus appears unlikely that PAF plays a major role in mediating glomerular functional alterations in this model of chronic glomerular inflammation. It remains possible, however, that its extracellular degradation rate (PAF-acetyl hydrolase activity) is altered in nephritic glomeruli as compared to controls.

The significant fall in protein excretion in group III, despite normalization of GFR, reflects a true improvement in glomerular sieving function in this group of animals. The lack of a similar improvement in group IV implicates non-TxA_2 products of the CO pathway in mediating, in part, the alterations in glomerular permselectivity induced by the inflammatory lesion. A role for PGE_2 is proposed in this regard in view of the known capacity of this eicosanoid to amplify alterations in vascular permeability in other models of inflammation (43). Furthermore, it seems unlikely that the improvement in glomerular sieving function in these animals is due to hemodynamic factors (44). The failure of protein excretion to fall in group IV rats, despite the reduction in ΔP, would also argue against elevations in this parameter as being of importance in mediating permselectivity changes in accord with the heteroporous membrane model, as recently proposed by Yoshioka et al. (45).

In summary, we have demonstrated, in a rat model of chronic mesangioproliferative glomerulonephritis, enhanced glomerular synthesis of PGE_2, $PGF_{2\alpha}$, and TxA_2, but not PAF and that the principal alteration leading to reduction of GFR is a fall in the glomerular capillary ultrafiltration coefficient, K_f. This fall in K_f can be prevented by earlier synthesis inhibition, or receptor antagonism, of TxA_2. In addition, maintenance of glomerular perfusion in this model of injury is crucially dependent on the local generation of PGE_2, the inhibition of which is associated with renal vasoconstriction and the elevation of systemic arterial pressure. Our findings provide insight into the pattern of glomerular dynamics during chronic glomerulonephritis, and the role of locally generated bioactive lipids in mediating alterations in glomerular functions. The data provide a mechanistic rationale, and further impetus, for evaluating the role of TxA_2 receptor antagonists, or synthesis inhibitors, in human glomerulonephritis.

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