Glomerular Prostaglandin and Thromboxane Synthesis in Rat Nephrotoxic Serum Nephritis

EFFECTS ON RENAL HEMODYNAMICS

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ABSTRACT Glomerular arachidonate cyclooxygenation by isolated rat glomeruli was assessed in vitro in antiglomerular basement membrane (anti-GBM) antibody-induced glomerulonephritis by radioimmunoassay for prostaglandins (PG) and thromboxane. After a single intravenous injection of rabbit anti-rat GBM serum, we observed enhancement of glomerular thromboxane B_2 (TxB_2) synthesis as early as 2 to 3 h with smaller increments in $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthetic rates. On day 2 of the disease, the glomerular synthesis of TxB_2 and, to a lesser extent, $PGF_{2\alpha}$ and PGE_2 remained enhanced, whereas on days 8, 11, and 14, TxB_2 was the only prostanoid synthesized at increased rates. Glomerular TxB_2 synthesis correlated with the presacrifice 24-h protein excretion. 60 min after intravenous infusion of anti-GMB serum, glomerular filtration rate (GFR) decreased (0.66 ± 0.04 to 0.44 ± 0.03 ml/min per 100 g, $P < 0.05$), without a significant change in renal plasma flow (RPF): 1.97 ± 0.23 to 1.80 ± 0.23 ml/min per 100 g) and without a change in glomerular PG synthetic rates. At 2 h, GFR and RPF reached a nadir (0.25 ± 0.04 and 1.3 ± 0.1 ml/min per 100 g, respectively) coinciding with a fivefold increment in glomerular TxB_2. By 3 h GFR and RPF partially recovered to 0.43 ± 0.07 and 1.77 ± 0.20 ml/

min per 100 g, respectively, $P < 0.05$, despite further increments in TxB_2 synthesis. This recovery of GFR and RPF coincided with increments in vasodilatory PG, (PGE_2 and PGI_2). The thromboxane synthetase inhibitor OKY-1581 markedly inhibited platelet and glomerular TxB_2 synthesis and preserved GFR at 1, 2, and 3 h. Another thromboxane synthetase inhibitor, UK-38485, also completely inhibited platelet and glomerular TxB_2 synthesis and prevented decrements of GFR at 2 and 3 h. A cyclooxygenase inhibitor, ibuprofen, inhibited platelet TxB_2 and PGE_2 synthesis and significantly reduced glomerular PGE_2 but not TxB_2 synthesis. In the ibuprofen-treated rats, the partial recoveries of GFR and RPF at 3 h were attenuated. The in vitro glomerular TxB_2 synthesis correlated inversely with the presacrifice GFR and filtration fraction. These observations indicate that in anti-GBM nephritis there is enhanced synthesis of TxA_2 and PG in the glomerulus that mediate changes in renal hemodynamics.

INTRODUCTION

Nephrotoxic serum nephritis (NSN),[1] produced by the administration of heterologous antibody to glomerular basement membrane (GBM) is a well-established experimental model of glomerular immune injury re-

[1] *Abbreviations used in this paper:* C_{IN}, inulin clearance rate; C_{PAH}, PAH clearance rate; C20:4, arachidonic acid; GBM, glomerular basement membrane; GFR, glomerular filtration rate; NSN, nephrotoxic serum nephritis; NTS, nephrotic serum; PAH, p-aminohippuric acid; PG, prostaglandin; PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$, prostaglandins E_2, $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$, respectively. RPF, renal plasma flow; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

sulting in glomerulonephritis and the nephrotic syndrome (1). This model of glomerular injury is characterized by a reproducible sequence of morphologic and pathophysiologic events that render it suitable for biochemical studies.

The involvement of a variety of cellular (2, 3) and humoral (4) mediators has been demonstrated in experimental anti-GBM nephritis, with potential roles attributed to changes in glomerular permeability to macromolecules (5), effects on glomerular vasculature (6), and chemotaxis of leukocytes into the glomerulus (7). The renal glomerulus has recently been shown to oxygenate arachidonic acid enzymatically through the cyclooxygenase (8) and the lipoxygenase (9) pathways to prostaglandins (PG), thromboxane, and hydroxylated fatty acids. These compounds have potent vasoactive (10) and chemotactic (11) properties and therefore could mediate, at least partially, pathophysiologic changes occurring in the course of experimental anti-GBM disease (12).

Our study evaluates the arachidonate cyclooxygenation and PG synthetic patterns in glomeruli isolated from rats with NSN. Furthermore, we assessed the pathophysiologic role of glomerular prostanoids, and in particular thromboxane A_2 (TxA_2), as mediators of the renal hemodynamic changes after administration of anti-GBM serum.

METHODS

Preparation and testing of anti-rat GBM serum. Anti-GBM serum was produced by immunization of three male albino rabbits with rat particulate GBM prepared from frozen rat kidneys (Pel-Freeze Biologicals, Inc., Rogers, AR) by a modification of the method of Krakower and Greenspon (13). The rabbits received six bimonthly injections (intradermally) of 5 mg rat GBM in complete Freund's adjuvant. Nephrotoxicity of rabbit sera was assessed at various time intervals: during the period of immunization, by the development of acute proteinuria after injection of 1 ml serum i.v. in male Sprague Dawley rats, and by morphologic evaluation of glomeruli under light, immunofluorescence, and electron microscopy.

When rabbit anti-rat GBM sera attained a sufficient titer to produce nephrotoxic lesions, the sera were pooled, complement inactivated at 56°C for 30 min, and absorbed with rat peripheral blood cells. The immune gamma globulin fraction, corresponding to 1 ml of pooled rabbit serum, was then estimated after gamma globulin isolation from an aliquot of the pool by the DEAE-cellulose method of Stanworth (14). In all subsequent experiments, 1 ml of rabbit anti-rat GBM serum (nephrotoxic serum, NTS) was employed.

Induction of anti-GBM disease. Male Sprague-Dawley rats, 250–300 g, were kept in metabolic cages with free access to standard diet and water. On day 0, anti-GBM disease was induced by the intravenous administration of 1 ml of rabbit anti-rat GBM serum, corresponding to a gamma globulin dose of 63–78 μg/100 g body wt. Control rats received 1 ml of serum obtained from rabbits immunized solely with complete Freund's adjuvant. The course of the NSN was evaluated over a 14-d period by daily determinations of 24-h urine protein excretion (15) and glomerular morphology according to standard methods for light, immunofluorescence, and electron microscopy.

Glomerular biochemical studies. On days 2, 8, 11, and 14 after administration of the NTS, rats were nephrectomized and the kidneys were placed in phosphate-buffered saline containing 150 mM NaCl, 10.5 mM Na_2HPO_4, and 3.8 mM NaH_2PO_4 (pH 7.4) at 4°C. Cortices from right and left kidneys were dissected from the medullae and processed separately for glomerular isolation; thus, two observations were obtained in each animal. After mincing of the cortices to a pastelike consistency, glomeruli were isolated by a technique previously used in this laboratory for glomerular biochemical studies (8). The minced cortical tissue was initially passed through a 106-μm metal sieve to remove large tubular fragments and the glomeruli were subsequently trapped on a 75-μm sieve. The purity of glomerular yields was determined microscopically, by counting the number of glomerular and nonglomerular particles suspended in a small volume of phosphate-buffered saline, and was found to vary between 88 and 95%. Glomeruli were generally devoid of Bowman's capsule and arterioles.

Isolated glomeruli were then suspended in preweighed, siliconized glass culture tubes in modified Earle's balanced salt solution, pH 7.4, containing gelatin 0.01 mg/ml, 25 mM *n*-2-hydroxyethylpiperazine-*n*'-2-ethanesulfonic acid (Hepes), and 5,8,11,14-eicosatetraenoic acid (arachidonic acid (C20:4), Sigma Chemical Co., St. Louis, MO) at a concentration of 5 μg/ml. Stock solutions of C20:4 dissolved in hexane at a concentration of 5 mg/ml, were kept at −35°C and were generally used within less than 1 mo of storage. Before each use, a 50-μl aliquot of stock C20:4 solution was dried under N_2 and 50 μl absolute ethyl alcohol was added. 5 μl of this solution were added in 5 ml modified Earle's solution, thus yielding a concentration of 5 μg/ml C20:4 and 0.1% ethanol. Glomeruli were then incubated in a volume of 1.5 ml media at 37°C for 45 min, without agitation. At the end of incubation, the glomeruli were centrifuged at 3,000 rpm for 10 min, the supernates were removed and frozen, and the wet weight of glomerular pellets was determined. Glomerular cyclooxygenation of C20:4 was assessed by determination of the major immunoreactive products of the cyclooxygenase pathway in the glomerular incubates, namely prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), thromboxane B_2 (TxB_2) (the stable degradation product of TxA_2) and 6-keto-prostaglandin $F_{1\alpha}$ (-$PGF_{1\alpha}$) (the stable hydrolysis product of PGI_2). Incubation media were assayed without prior extraction. Antiserum for PGE_2 was purchased from the Institute Pasteur, Paris (16). Rabbit antisera for $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were prepared in our laboratory. Anti-TxB_2 serum was a gift from Dr. William Campbell, Dallas, TX. Tritiated ligands of all four prostaglandins were obtained from New England Nuclear, Boston, MA. On several occasions, ^{125}I-histamine-6-keto-$PGF_{1\alpha}$ and ^{125}I-histamine-TxB_2 were used as ligands for the radioimmunoassays (RIA) of 6-keto-$PGF_{1\alpha}$ and TxB_2. These were made in our laboratory by the method of Maclouf et al. (17). The sensitivities and cross-reactivities of these four RIA have been previously described (18). Glomerular PG synthetic rates were expressed as picograms of immunoassayable PG per milligram glomerular wet weight per 45-min incubation.

Incubation media free of glomeruli but containing 5 μg/ml C20:4 were also assayed for all four PG in order to account for the cross-reactivity of PG antisera with C20:4.

Renal perfusion studies. In four rats, on day 2 after administration of NTS, the right kidneys were removed immediately after ligation of the right renal artery, while the

remaining left kidneys were perfused in situ through the abdominal aorta, with modified Earle's solution (50–75 ml), until they became completely blanched and free of blood. The effect of perfusion on glomerular cellularity was assessed by light-microscopic examination of serial cortical sections. Glomeruli were then isolated from perfused and nonperfused kidneys for biochemical studies as described above. In this manner, the approximate contribution of blood-borne cells on glomerular arachidonate cyclooxygenation was assessed.

Renal clearance, thromboxane synthetase inhibition, and cyclooxygenase inhibition studies. The effect of NTS on renal hemodynamics—glomerular filtration rate (GFR) and renal plasma flow (RPF)—and their modification by thromboxane synthetase inhibition and cyclooxygenase inhibition was studied by inulin and *p*-aminohippuric acid (PAH) clearance methods. Male Sprague-Dawley rats (250–300 g) were anesthetized by intraperitoneal injection of Inactin, 16 mg/ 100 g body wt, tracheostomized and placed on a constant temperature table. GFR and RPF were determined by the clearance of inulin (Polyfructosan-Inutest, Laevosan-Gesellschaft, Linz/Donau, Austria) and PAH (Eastman Kodak Co., Rochester, NY) respectively. 200 mg PAH was dissolved in 20 ml Inutest (0.25 g/inulin per ml). 900 mg NaCl was added and the final volume was brought up to 100 ml with distilled water. Thus, the final solution contained 0.2% PAH and 5% inulin. 1.5 ml of this solution was infused in the internal jugular vein as a priming load, followed by constant infusion of the same solution via a Razel pump (Razel Scientific Instruments, Inc., Stamford, CT) at 3 ml/min. A 60-min period of constant infusion was allowed before initiation of clearances to attain constant plasma levels of inulin and PAH. 70-μl blood samples were obtained from the tail vein at the midpoint of each clearance period. Urine was collected in preweighed vessels via a large-bore polyethylene tube inserted in the bladder via a small incision at the fundus. Plasma and urine inulin and PAH were determined by methods previously described (19, 20), modified for microliter samples.

In experiments involving infusions of NTS and/or inhibitors, arterial blood pressure was monitored constantly via a polyethylene tubing (PE-10) inserted in the femoral artery and connected to a Gould brush 220 recorder (Gould Instruments Div., Gould, Inc., Cleveland, OH) via a carrier amplifier. Each experiment involved four clearance periods. The first 40-min clearance preceded administration of NTS. The second 40-min clearance period was initiated 20 min after the intravenous infusion (>4–5 min) of 1 ml NTS and was followed by a third and fourth clearance, each of 60 min duration. At the end of the fourth clearance period, rats were nephrectomized and glomeruli were isolated and processed for biochemical studies as described above. In a group of rats, clearances were terminated and glomerular studies were performed at 1 (*n* = 6), 2 (*n* = 4), and 3 h (*n* = 4) after administration of NTS. Control rats received 1 ml of nonnephrotoxic serum from rabbits injected only with Freund's adjuvant. Thus, the time sequence of glomerular arachidonate cyclooxygenation and PG synthetic profiles was assessed in parallel with pathophysiologic changes.

In 13 thromboxane synthetase inhibition experiments, the inhibitor sodium (E)-3-(4-(3-pyridylmethyl)phenyl)-2-methyl acrylate, also known as OKY-1581, (Ono Pharmaceutical Co., Osaka, Japan) was infused along with the inulin-PAH solutions for 100 min before and during the administration of NTS and throughout all three clearance periods after NTS infusion. A loading dose of OKY-1581, 150 μg, dissolved in the priming inulin-PAH solution, was followed

by varying amounts of the inhibitor infused constantly with the sustaining inulin-PAH solution. In all inhibition experiments, the loading dose was kept constant, while three different sustaining infusion rates of the inhibitor were used: 150 (*n* = 3), 450 (*n* = 4), and 1,500 (*n* = 6) μg/h. Clearance and postsacrifice glomerular prostaglandin values were compared with parallel experiments (*n* = 8) using NTS infusions alone.

In four additional thromboxane synthetase inhibition experiments, the effect of the inhibitor 3-(1H-imidazol-1-ylmethyl)-2-methyl-1H-indole-1-propanoic acid, also known as UK-38485, (Pfizer Chemicals Div., Pfizer Inc., Groton, CT) on renal hemodynamic changes induced by NTS was evaluated. The mode of administration of this inhibitor was similar to that of OKY-1581, namely a loading dose of 150 μg i.v. followed by a constant infusion rate of 400 μg/h. Results were compared with four experiments in which NTS was infused alone. The effect of the cyclooxygenase inhibitor ibuprofen on NTS-induced changes in GFR and effective RPF was evaluated in four clearance experiments. Ibuprofen, (Upjohn Co., Kalamazoo, MI) dissolved in 0.1 M sodium diphosphate buffer, was administered i.v., 13 mg/kg body wt, 40–60 min before NTS infusion and i.p., 13 mg/kg body wt immediately after NTS infusion. Results were compared with four experiments in which NTS was infused without ibuprofen. In the UK-38485 and ibuprofen experiments GFR and RPF were measured with infusions of [³H]inulin and [¹⁴C]PAH (New England Nuclear, Boston, MA).

The effect of OKY-1581, UK-38485, and ibuprofen on nonrenal (platelet) TxA₂ synthesis was assessed throughout the clearance experiments, before, and at hourly intervals after, NTS administration. 200 μl venous blood obtained at midpoint of each clearance period was allowed to clot at 37°C for 30 min and subsequently centrifuged at 3,000 rpm for 15 min (21). Unextracted sera were assayed for TxB₂ and expressed as nanograms of TxB₂ per milliliter serum. Immunoassayable glomerular TxB₂ was determined along with the other PG after incubation with C20:4 as described above.

For statistical comparisons, the analysis of variance and Student's *t* test were used for paired and unpaired observations as indicated by the experimental design (22). Linear regression analysis was used to correlate glomerular TxB₂ synthesis with urinary protein excretion, GFR, and filtration fraction.

RESULTS

Glomerular morphologic changes and prostaglandin synthetic profiles after NTS infusion. After administration of NTS, proteinuria, ranging from 46 to 235 mg/24 h, developed in all rats within <12 h. Glomerular morphology at day 2 revealed changes consistent with the heterologous phase of anti-GBM disease (1). These changes included linear deposition of rabbit IgG on rat GBM, proliferation of mesangial and endothelial cells, focal areas of polymorphonuclear and mononuclear cell infiltration, and detachment of the glomerular endothelial lining at multiple sites (Fig. 1 *a* and *b*). On day 14, after injection of NTS, glomerular morphology was consistent with changes occurring during the autologous phase of the disease. These included linear deposition of rabbit IgG, rat IgG, and C₃; focal areas of glomerular sclerosis; and almost
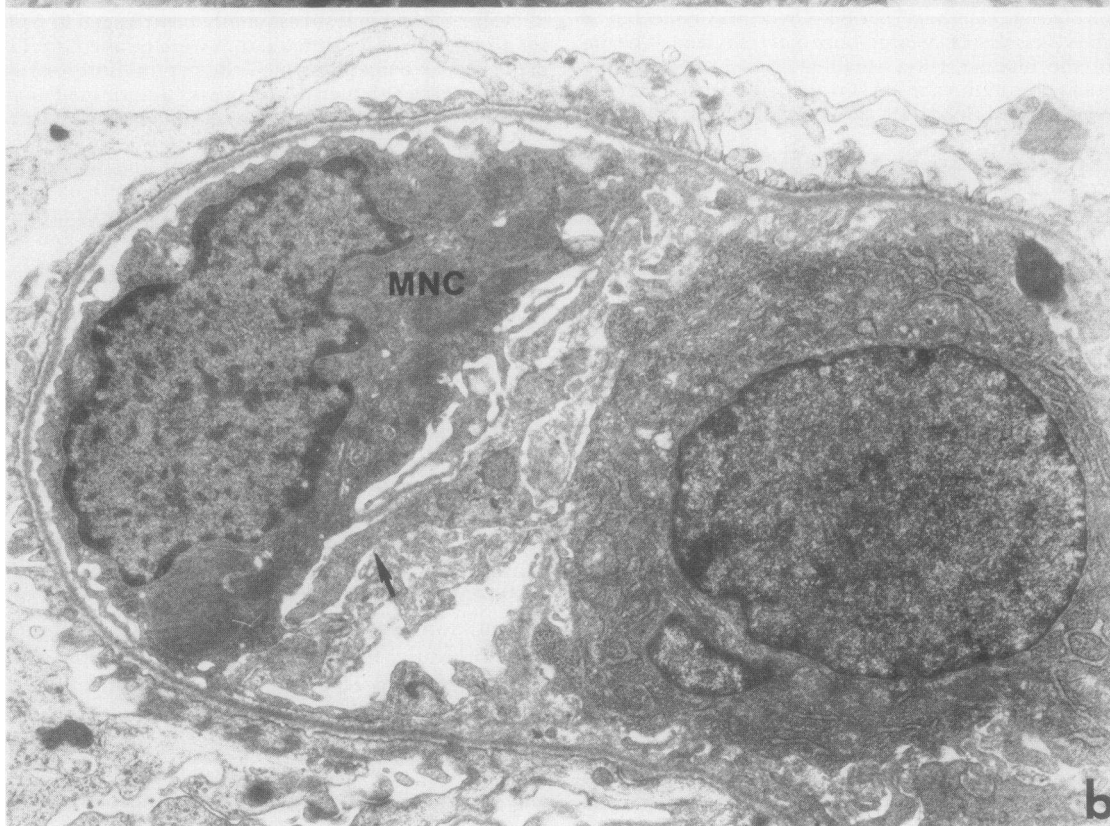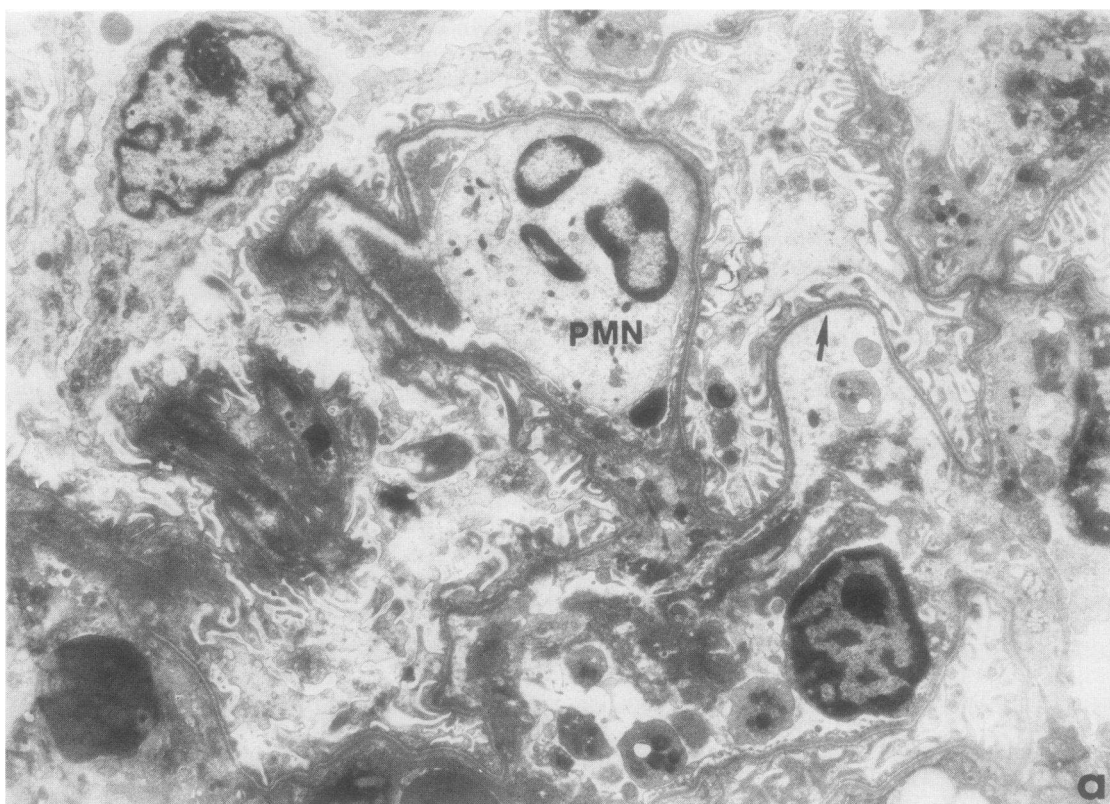
complete detachment of the endothelium from the GBM. Areas of mesangial cell proliferation and mononuclear cell infiltration were also seen.

The glomerular synthetic rates of PG and TxA_2 during the heterologous (day 2) and the autologous phases (days 8, 11, and 14) are summarized in Fig. 2. On day 2 after induction of NSN, there was a 10-fold increment of glomerular TxA_2 release, with smaller increases in the synthesis of PGE_2 and $PGF_{2\alpha}$ (4- and 2.3-fold respectively). On days 8, 11, and 14, only glomerular TxA_2 synthesis remained enhanced.

To assess the contribution of circulating cells, especially platelets and leukocytes on glomerular arachidonate cyclooxygenation, kidneys were perfused free of blood on day 2 in four control and four nephritic rats. Renal perfusion had no effect on glomerular PG and TxA_2 synthesis in glomeruli from control or NSN rats, suggesting no significant contribution of blood-borne cells entrapped in glomerular capillaries.

To assess the possible association of glomerular TxA_2 synthesis with glomerular injury, TxB_2 synthetic rates were correlated with the urinary protein excretion. A significant correlation ($r = 0.66$, $P < 0.05$, $n = 13$) was found between glomerular TxB_2 synthesis and urinary protein excretion determined in the 24-h collections just before the animal was killed (Fig. 3). Glomerular $PGF_{2\alpha}$ and PGE_2 synthesis did not correlate with protein excretion.

Acute effects of NTS on renal hemodynamics and glomerular PG synthesis. Fig. 4 depicts the sequence of acute changes in GFR and RPF, after NTS, in parallel with changes in glomerular PG synthesis, determined in vitro, and expressed as fold-increments compared with simultaneous controls (NTN/control). At 1 h, there was a significant decrement in the inulin clearance rate (C_{IN}) (0.66±0.04 to 0.44±0.03 ml/min per 100 g, $P < 0.05$), accompanied by an insignificant change in the PAH clearance rate (C_{PAH}) (1.97±0.23 to 1.80±0.23 ml/min per 100 g). Glomerular PG synthesis was unaltered compared with simultaneous controls. At 2 h, C_{IN} further decreased to 0.25±0.04 ml/min per 100 g, $P < 0.01$ compared with the preceding hour and C_{PAH} fell to 1.30±0.1 ml/min per 100 g, $P < 0.05$. The synthesis of TxB_2 and $PGF_{2\alpha}$ were enhanced by 4.6±0.1- ($P < 0.0001$) and 1.7±0.5-fold ($P < 0.05$), respectively. At 3 h, there was a significant recovery of C_{IN} to 0.43±0.07 ml/min per 100 g, ($P < 0.05$) and C_{PAH} to 1.77±0.20 ml/min per 100 g (P
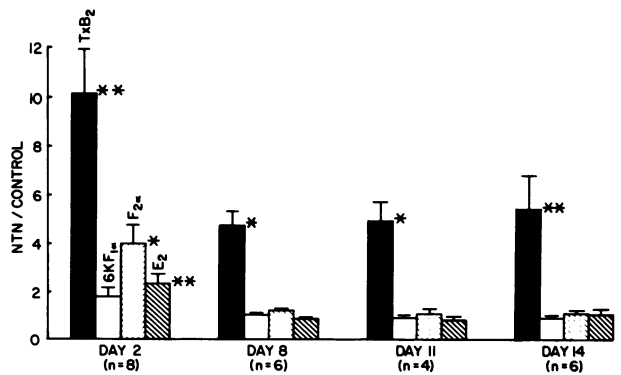

FIGURE 2 Glomerular prostaglandin synthetic patterns at various times over a 14-d period after a single administration of NTS. Each bar represents the fold-increment of a given prostaglandin compared with control. PG synthesis in control glomeruli, in nanograms per milligram glomerular weight per 45 min ($n = 24$), were: $TxB_2 = 35\pm3$; $PGF_{2\alpha} = 810\pm76$; $PGE_2 = 104\pm8$; 6-keto-$PGF_{1\alpha} = 56\pm3$. *, $P < 0.005$; **, $P < 0.0001$.

< 0.05). At 3 h, TxB_2 synthesis was further increased to 11±0.4-fold ($P < 0.001$), while glomerular $PGF_{2\alpha}$, PGE_2, and 6-keto-$PGF_{1\alpha}$ synthesis were also enhanced by 4.7±0.4- ($P < 0.001$) 3.7±0.8- ($P < 0.01$), and 2.6±0.4-fold ($P < 0.01$), respectively.

Modification of NTS-induced changes in renal hemodynamics by thromboxane synthetase inhibition and cyclooxygenase inhibition. The effects of OKY-1581, UK-38485, and ibuprofen were assessed on glomerular and nonrenal (platelet) thromboxane synthesis. Platelet TxB_2 release after whole-blood coagulation for 30 min at 37°C was determined in all clearance experiments in which the above inhibitors were used. All three inhibitors reduced platelet TxB_2 by 95–97% (248±54 to 6±1.3 ng/ml serum, $n = 21$) before NTS infusion and this degree of inhibition was maintained throughout the subsequent three clearance periods. Ibuprofen also produced a sustained 97% inhibition of serum PGE_2 (28.2±1.5 to 0.76±0.23 ng/ml serum).

Effects of OKY-1581 (Fig. 5). At infusion rates of 150 and 450 μg/h, OKY-1581 did not uniformly inhibit glomerular thromboxane synthesis (synthetic rates: 131–1,062 pg/mg glomerular wt per 45 min) and did not alter NTS-induced changes in C_{IN} or filtration fraction compared to experiments using NTS alone. When OKY-1581 was infused at rates of 1,500 μg/h ($n = 6$),

FIGURE 1 (a) Glomerulus at 3 h after injection of anti-GBM serum. The capillary lumen is obliterated by a *PMN*. The endothelium of the capillary (arrow) is missing. × 4,000. (b) Glomerulus at day 2 after injection of anti-GBM serum. The lumen of the capillary is obliterated by two mononuclear cells. The cell on the right has abundant endoplasmic reticulum and might be endothelial or blood-borne. The cell on the left is a monocyte (*MNC*) that has penetrated between the lifted endothelium (arrow) and the naked basement membrane × 8,000.
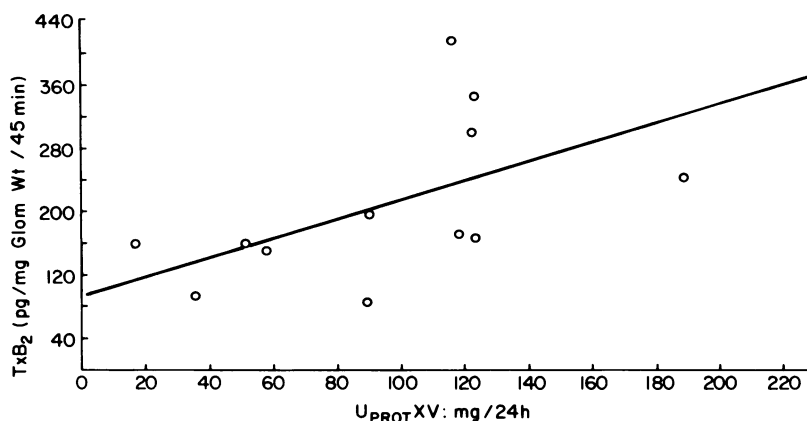
FIGURE 3 Correlation between the presacrifice 24-h urinary protein excretion ($U_{PROT}XV$) and the postsacrifice glomerular TxB_2 synthetic rates in animals studied between 2 and 14 d after a single administration of NTS. $n = 13$; $r = 0.66$, and $P < 0.05$.

glomerular TxB_2 synthesis was uniformly inhibited (TxB_2 synthetic rates: 14–33 pg/mg glomerular wt per 45 min). The changes of C_{IN} at 1, 2, and 3 h after NTS infusion ($n = 8$) and their modification by OKY-1581 infused at a loading dose of 150 μg, followed by 1,500 μg/h ($n = 6$), are shown in Fig. 5. Glomerular PG synthetic profiles, determined in vitro after termination of each clearance experiment, are also shown. Constant infusions of OKY-1581 for a period of 100 min before NTS administration (equilibration time plus pre-NTS clearance) had no significant effect on C_{IN} (0.73±0.06 compared with 0.75±0.08 ml/min 100 g wt). After NTS, C_{IN} fell to 0.44±0.04 ml/min per 100 g, $P < 0.05$ at 1 h and reached a nadir (0.28±0.06

ml/min per 100 g, $P < 0.005$) at 2 h. At 3 h, C_{IN} recovered significantly to 0.43±0.04 ml/min per 100 g, $P < 0.05$, but remained depressed compared to pre-NTS values (0.43±0.04 compared with 0.73±0.06 ml/min per 100 g, $P < 0.05$). In the OKY-1581-treated group C_{IN} fell only at 2 h after NTS (0.75±0.08 to 0.54±0.08 ml/min per 100 g, $P < 0.05$). When compared with the untreated group, C_{IN} was significantly higher at all time points after NTS infusion in the OKY-1581-treated rats. OKY-1581 markedly inhibited glomerular TxB_2 synthesis (23±2 compared with 1029±104 pg/mg glomerular wt per 45 min). Glomerular PGE_2 synthesis was also reduced (210±26 compared with 655±76 pg/mg glomerular wt, $P < 0.001$), whereas there were no significant changes in $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ production between the two groups (Fig. 5). There was no significant change in filtration fraction at 1 h after NTS in the untreated group, but it fell to 0.18±0.03 at 2 h ($P < 0.005$ compared with 0.36±.04, pre-NTS) and remained depressed at 3 h (0.19±0.03, $P < 0.05$ compared with
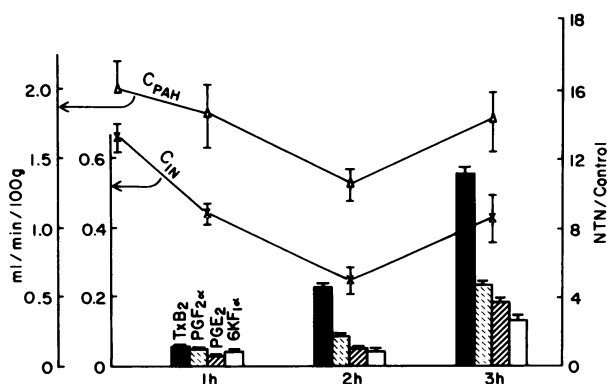

FIGURE 4 Acute changes in C_{IN} and C_{PAH}, left ordinates, and changes of glomerular prostaglandin synthesis expressed as fold-increments compared with simultaneous controls, right ordinate. PG synthesis in control glomeruli, in nanograms per milligram glomerular weight per 45 min ($n = 12$), were: $TxB_2 = 79\pm2$; $PGF_{2\alpha} = 1,199\pm28$; $PGE_2 = 550\pm81$; 6-keto-$PGF_{1\alpha} = 46\pm3$.
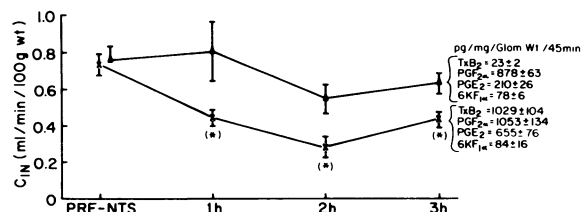

FIGURE 5 Modification of NTS-induced changes in GFR by OKY-1581. (Δ) OKY-1581 plus NTS, $n = 6$; ($\times$) NTS, $n = 8$. At infusion rates of 1,500 μg/h, OKY-1581 markedly inhibited glomerular TxB_2 synthesis and preserved GFR. PGE_2 synthesis was also decreased. See text for statistical comparisons. *, $P < 0.05$.

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pre-NTS). In the OKY-1581-treated rats, filtration fraction was preserved at 2 and 3 h after NTS administration.

Effects of UK-38485 (Fig. 6). UK-38485, infused at 400 μg/h, did not prevent decrements in C_{IN} at 1 h after administration of NTS (0.97 ± 0.2 compared with 0.88 ± 0.15 ml/min per 100 g body wt, $P > 0.05$). At 2 and 3 h, C_{IN} values were significantly higher in the UK-38485-treated group compared with those obtained in control animals receiving NTS alone (1.04 ± 0.13 compared with 0.62 ± 0.15 ml/min per 100 g body wt, $P < 0.05$, at 2 h and 1.12 ± 0.04, compared with 0.84 ± 0.13 ml/min per 100 g body wt, $P < 0.05$ at 3 h). In this group,C_{PAH} and filtration fraction values were higher, but not significantly different, compared with NTS controls. In vitro glomerular TxB_2 synthesis was uniformly inhibited in the UK-38485-treated group, whereas PGE_2 and 6-keto-$PGF_{1\alpha}$ synthetic rates were not altered (Fig. 6).

Effects of ibuprofen (Fig. 7). Ibuprofen, a cyclooxygenase inhibitor, administered i.v., 13 mg/kg, and i.p., 13 mg/kg, did not prevent the decrements of GFR and RPF at 1 and 2 h after NTS infusion. In the rats receiving ibuprofen, the partial recovery in GFR at 3 h was attenuated compared with NTS control animals (0.62 ± 0.03 compared with 0.88 ± 0.12 ml/min per 100 g body wt, $P < 0.05$) (Fig. 7). Ibuprofen also blocked the partial recovery in C_{PAH} (1.96 ± 0.37 compared with 2.77 ± 0.18 ml/min per 100 g body wt, $P < 0.05$, not shown). Despite adequate serum thromboxane inhibition, ibuprofen did not inhibit glomerular TxA_2 synthesis compared with NTS control animals. In fact, glomerular TxB_2 values were significantly higher in the ibuprofen-treated group (985 ± 120 compared with 840 ± 101 pg/mg glomerular wt, $P < 0.005$) and glomerular PGE_2 and 6-kto-$PGF_{1\alpha}$ were significantly lower than in NTS controls (PGE_2: 484 ± 59 compared with $1,044\pm116$ pg/mg glomerular wt, P

FIGURE 7 Effects of ibuprofen on NTS-induced changes in GFR. (X) NTS, $n = 4$. (O) ibuprofen plus NTS, $n = 4$. Ibuprofen, at a total dose of 26 mg/kg wt did not protect GFR at 1 and 2 h and attenuated partial recovery of GFR at 3 h. Glomerular $PGE_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ synthesis were significantly reduced, while that of TxB_2 was significantly higher in the NTS group.

< 0.05; 6-keto-$PGF_{1\alpha}$: 219 ± 19 compared with 336 ± 30 pg/mg glomerular wt).

In Fig. 8, C_{IN} in the hour before the animal was killed is correlated with in vitro glomerular TxA_2 synthetic rates in all clearance experiments ($n = 22$) involving administration of NTS alone and NTS with OKY-1581 at three different infusion rates (150, 450, and 1,500 μg/h). A significant inverse correlation was found ($r = 0.67$, $P < 0.01$). An inverse correlation was also found between glomerular TxB_2 synthesis and filtration fraction ($r = 0.57$, $P < 0.05$, data not shown).
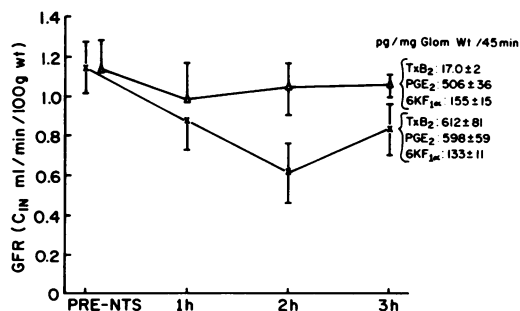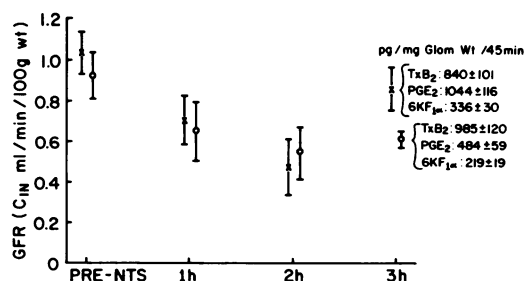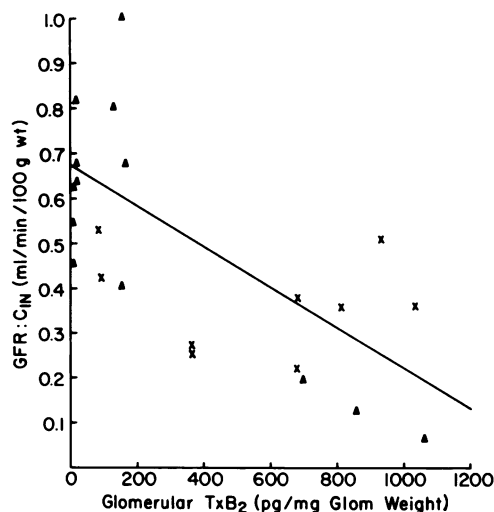

FIGURE 6 Modification of NTS-induced changes in GFR by UK-38485. (X) NTS, $n = 4$. (Δ) UK-38485 plus NTS, $n = 4$. At infusion rates of 400 μg/h, UK-38485 inhibited glomerular TxB_2 synthesis and preserved GFR at 2 and 3 h. Synthesis of PGE_2 and 6-keto-$PGF_{1\alpha}$ were unaltered.

FIGURE 8 Correlation between GFR and in vitro glomerular TxB_2 synthesis. C_{IN} in the hour before the animal was killed was correlated inversely with glomerular TxB_2 synthesis in all experiments involving administration of NTS without (X) and with (Δ) OKY-1581 infusion at three different rates (150, 450, and 1,500 μg/h), thereby resulting in different degrees of glomerular TxB_2 inhibition. $n = 22$; $r = -0.67$; $P < 0.01$.

DISCUSSION

NSN, induced by administration of anti-GBM antibody, is associated with a spectrum of pathophysiologic changes and involves a variety of cellular and humoral mediators in the host. Systems that are thought to mediate or induce glomerular structural and functional damage include polymorphonuclear leukocytes (PMN) and monocytes (3, 23), platelets (24), the complement system (25), the Hageman factor system (26), and vasoactive amines (6).

A potential role of C20:4 metabolites in glomerular immune injury can be reasoned on the basis of several lines of evidence: (a) Cyclooxygenase inhibition has been shown to decrease urinary protein excretion, particularly when combined with volume depletion, in patients with various forms of immunologically mediated glomerulopathies (27). (b) Isolated glomeruli (8), glomerular epithelial cells (28), and glomerular mesangial cells (29) are capable of cyclooxygenating C20:4 to $PGF_{2\alpha}$ and PGE_2 and to a lesser extent TxA_2 and 6-keto-$PGF_{1\alpha}$. (c) Cells (leukocytes, monocytes, platelets) invariably present in the course of anti-GBM disease and thought to be involved in mediating glomerular injury synthesize PG and TxA_2 (30–32).

The results of our study indicate that enhanced arachidonate cyclooxygenation by isolated glomeruli commences as early as 2 h after administration of NTS (Fig. 4). The synthesis of TxB_2 and $PGF_{2\alpha}$ were increased within 2 h, whereas PGE_2 and 6-keto-$PGF_{1\alpha}$ were enhanced after 3 h. On day 2, TxB_2, $PGF_{2\alpha}$, and PGE_2 continued to be produced at increased rates, with TxB_2 showing the greatest increment. Throughout the rest of the 2-wk course, namely on days 8, 11, and 14, only TxB_2 synthesis remained enhanced (Fig. 2).

Several sources of PG and TxA_2 synthesis can be implicated in this model of glomerular immune injury. Blood-borne cells (leukocytes, monocytes, platelets) recruited to sites of antigen-antibody interaction and complement activation along the GBM, could account for the enhanced PG and TxA_2 synthetic rates. In experimental anti-GBM disease, when complement-fixing anti-GBM antibodies are used, there is an influx of PMN, eventually replaced by mononuclear cells (33). Light and electron microscopic evaluation of glomerular cellularity (Fig. 1 a) demonstrated the presence of PMNs within 3 h after NTS infusion. An endogenous glomerular source of prostanoids is also possible, since both epithelial and mesangial cells are capable of synthesizing PG and thromboxanes (28, 29, 34). Mesangial cell proliferation is a common feature of nephrotoxic glomerulonephritis and was seen during the heterologous phase in all animals studied. Furthermore, mesangial cells have been reported to be involved in glomerular morphologic changes as early

as 20 min after injection of NTS, with mesangial cell interposition between endothelial cells and GBM occurring before the PMN influx (35).

Evidence that the enhanced glomerular PG and TxA_2 synthesis may not be entirely from blood-borne cellular sources includes the following observations: (a) Renal perfusion to remove nonadherent cells decreased glomerular cellularity, assessed histologically, and did not significantly reduce PG and TxA_2 synthesis in NSN glomeruli compared with contralateral nonperfused NSN controls. (b) OKY-1581 at 150 and at 450 μg/h induced a 97% inhibition of serum (primarily platelet) TxA_2, but did not uniformly inhibit glomerular TxA_2 synthesis. (c) Ibuprofen had no effect on glomerular TxA_2 synthesis despite a 95–97% inhibition of serum TxA_2. It should be pointed out, however, that both perfusion techniques and pharmacologic maneuvers might be ineffective in removing or inhibiting blood-borne cells that have migrated into the mesangium.

The anti-GBM antibody induced changes in renal and glomerular hemodynamics have been studied by several groups. These investigators have assessed both the immediate (45–60 min) and the late (5–20 mo) effects of anti-GBM sera (12, 36, 37). A key feature of NTS-induced hemodynamic changes is reduction in whole kidney and single nephron GFR, attributable primarily to decrements in ultrafiltration coefficient and in single nephron RPF, the latter being dependent on the amount of immune globulin infused (38).

In our studies, NTS reduced the GFR as early as 1 h with a nadir at 2 h and partial recovery by 3 h. Glomerular TxA_2 was normal at 1 h and was increased 4.6- and 11-fold at 2 and 3 h, respectively. To evaluate the pathophysiologic importance of TxA_2 in this NSN model, we used two different inhibitors of TxA_2 synthetase. OKY-1581 is a pyridine derivative which inhibits TxA_2 production in vitro (39) and in vivo (40). UK-38485, an imidazole compound, is also a potent TxA_2 inhibitor (41). Neither drug inhibits cyclooxygenase at doses necessary to decrease TxA_2 (39–41). In our experiments both OKY-1581 (1,500 μg/h) and UK-38485 (400 μg/h) completely blocked the increment of glomerular TxA_2 after induction of NSN. Biochemical inhibition of TxA_2 synthesis, in glomeruli, prevented the expected decrements of GFR, RPF and filtration fraction at 2 and 3 h after NTS. OKY-1581, but not UK-38485, also improved the GFR at 1 h, perhaps through pharmacologic actions apart from TxA_2 inhibition, since glomerular TxA_2 was normal at 1 h. Blantz et al. (42) have postulated that alpha adrenergic stimulation accounts for the decrements of GFR 45–60 min after administration of NTS. The partial recovery of GFR at 3 h occurred despite further increments in glomerular TxA_2 synthesis. This recov-

ery coincided with significant increments in the synthesis of glomerular PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$. Ibuprofen attenuated the partial recovery in GFR and effective RPF (Fig. 7). Although used in large doses, ibuprofen failed to decrease glomerular TxA_2 synthesis, whereas it did reduce the synthetic rates of PGE_2 and 6-keto-$PGF_{1\alpha}$. These observations support a role for these vasodilatory PG in mediating the partial recovery in GFR and RPF at 3 h after NTS infusion.

Potential sites of action of TxA_2 on glomerular vasculature are the afferent and efferent arterioles, which have increased resistance after administration of anti-GBM serum (38). Since in our studies GFR decreased more than RPF, an effect of thromboxane on the factors regulating glomerular filtration could be postulated. Of these factors, the ultrafiltration coefficient, which is the product of filtration surface area and hydraulic conductivity, appears to be particularly sensitive to the effects of anti-GBM serum (36, 38). A decrement in hydraulic conductivity after NTS infusion has been postulated (36) on the basis of detachment of the endothelial lining commonly observed, thereby creating an unstirred layer. On the other hand, changes in filtration surface area could result from changes in contractility of the glomerular mesangial cells (43, 44). Endoperoxide analogues, which mimic the actions of TxA_2, are potent constrictors of isolated glomeruli (Scharschmidt, L. S., and Dunn, M. J., unpublished observations). We speculate that enhanced TxA_2 may induce changes in the contractile state of the glomerular mesangial cells and thereby reduce the filtration surface.

This study has characterized the glomerular PG synthetic profiles in a model of experimental glomerulonephritis. It appears that arachidonate cyclooxygenation products, particularly TxA_2, must be added to the list of important nonimmune mediators in this model of glomerular immune injury. An important pathophysiologic role of TxA_2 in the renal hemodynamic changes of NSN was demonstrated and other roles (proaggregatory, proadhesive) might be expected in the course of glomerulonephritis. Characterization of the arachidonate pathway(s) in this and other models of glomerular immune injury may allow pharmacologic manipulations and possibly therapeutic intervention without particular regard to the precise type of antigen(s) or antibody involved. Finally, glomerular prostanoids, and particularly TxA_2, may prove to serve as useful markers of glomerular immune injury.

ACKNOWLEDGMENTS

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