Increases in Glomerular Eicosanoid Production in Rats with Bilateral Ureteral Obstruction Are Mediated by Enhanced Enzyme Activities of Both the Cyclooxygenase and 5-Lipoxygenase Pathways (43601)

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Abstract. **Glomeruli isolated from rats with bilateral ureteral obstruction (BUO) of 24 hr duration produced significantly greater amounts of prostaglandin (PG) E_2, 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and leukotriene B_4 than glomeruli isolated from sham-operated control (SOC) rats. To examine the mechanisms underlying the greater production of eicosanoids by glomeruli isolated from rats with BUO, we measured the activities of enzymes related to eicosanoid formation such as cyclooxygenase, 5-lipoxygenase, PGE_2 isomerase, and PGI_2 and thromboxane synthase in glomeruli isolated from SOC rats and rats with BUO. Glomeruli isolated from rats with BUO had a significantly increased activity of cyclooxygenase with *de novo* synthesis of this enzyme and a markedly augmented activities of PGE_2 isomerase and both PGI_2 and thromboxane synthases relative to glomeruli isolated from SOC rats. Similarly, the activity of membrane-bound 5-lipoxygenase, the active location of this enzyme, was significantly greater in glomeruli isolated from rats with BUO than in glomeruli isolated from SOC rats. Thus, BUO of 24 hr duration enhances the glomerular production of eicosanoids via the activation of enzymes in both the cyclooxygenase and 5-lipoxygenase pathways.** [P.S.E.B.M. 1993, Vol 203]

Bilateral ureteral obstruction (BUO) leads to a marked decline in glomerular filtration rate (GFR) and renal plasma flow by 24 hr after the onset of obstruction (1). These functional changes are the consequence of increased vasoconstriction of glomerular arterioles and decreased ultrafiltration coefficient (K_f) due to glomerular mesangial cell contraction (1). The hormones, angiotensin II, thromboxane (Tx) A_2, and leukotriene (LT) B_4 are in part involved in the pathogenesis of this vasoconstrictive state and/or decreased K_f by mesangial cell contraction (1–4). Indeed, prior inhibition of these vasoconstrictors with the angiotensin-converting enzyme inhibitor, enalapril, the thromboxane synthase inhibitor, OKY-046, or the 5-lipoxygenase inhibitor, MK 886, improves the decrement in GFR and renal plasma flow seen at 24 hr after the onset of BUO (4, 5). By contrast, the vasodilatory prostaglandins, prostaglandin (PG) E_2 and prostacyclin, are increased to prevent further decrements in GFR and renal plasma flow by antagonizing the action of vasoconstrictors (1). We have recently described increased production of vasoactive eicosanoids including PGE_2, prostacyclin, TxA_2, and LTB_4 in glomeruli obtained from rats with BUO of 24 hr duration (2–4) that

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is accompanied by an enhanced renin-angiotensin system (1).

The greater production of prostaglandins and thromboxane by glomeruli from rats with BUO is mediated by angiotensin II-induced activation of phospholipase A_2 and cyclooxygenase (2, 3). To the best of our knowledge, it is not known whether BUO influences the activity of other enzymes involved in eicosanoid formation such as PGE_2 isomerase, thromboxane synthase, and prostaglandin I_2 synthase in rat glomeruli. McNamara *et al.* (6) and Fukuda *et al.* (7) evaluated the activity of PGE_2 isomerase, thromboxane synthase, and PGI_2 synthase in the whole kidney of rats with unilateral ureteral obstruction. They found that by 72 hr after the onset of obstruction, the experimented kidney had a significantly greater activity of thromboxane and PGI_2 synthase than the contralateral, untouched kidney. On the other hand, the activity of PGE_2 isomerase in the obstructed kidney was similar to that observed in the contralateral kidney (6, 7). Moreover, there was no significant difference in the activity of these three enzymes between the contralateral kidney of rats with unilateral ureteral obstruction and the kidney of sham-operated control (SOC) rats (7).

The present study was designed to determine the effects of BUO on the activities of PGE_2 isomerase, thromboxane synthase, and PGI_2 synthase. We have compared the activities of these enzymes in glomeruli from SOC rats and rats with BUO of 24 hr duration. In addition, we examined the activity of the membrane-bound 5-lipoxygenase that regulates the production of LTB_4 in glomeruli obtained from both groups of rats.

Materials and Methods

Chemicals and Reagents. Polyclonal cyclooxygenase antibody and prostaglandin H_2 were purchased from Cayman Chemical Co. (Ann Arbor, MI). Deoxyribonuclease I type II and arachidonic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase type II was purchased from Worthington Biochemical Corp. (Freehold, NJ). Bovine serum albumin, 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium were supplied by Wako Chemicals (Osaka, Japan).

Animal Models and Preparation of Isolated Glomeruli. Female Sprague-Dawley rats (200–250 g) purchased from Japan Biochemical Supplement Center (Tokyo, Japan) underwent sham operation (n = 5) or bilateral ureteral ligation (n = 5) under ether anesthesia as reported previously (2, 3, 8). The rats were then returned to cages and given no food and water. Twenty-four hours after operation, the abdominal cavity of the animals was opened under pentobarbital anesthesia (4 mg/100 g body wt) and both kidneys were harvested after perfusion with phosphate-buffered saline injected just above the bifurcation of the aorta. The kidneys were decapsulated on ice and glomeruli were isolated by pressing the cortical tissue through three consecutive sieves (mesh size: 250 μm, 150 μm, and 75 μm) (2, 3, 8). The glomeruli were then treated with 60 units/ml of collagenase type II and 0.03 mg/ml of DNase under continuous agitation (140 cycles/min) for 10 min at 37°C to remove Bowman's capsule. After treatment, the preparations were washed three times with cold Hanks' balanced salt solution and kept on ice for the subsequent procedure. Purity of the glomeruli was confirmed by light microscopy to be over 90% isolated glomeruli, most of which were free of Bowman's capsule.

Glomerular Eicosanoid Production. The glomerular biosynthesis of eicosanoids was examined with a slight modification as described previously (2, 3, 8). Isolated glomeruli were suspended in warm Hanks' balanced salt solution and preincubated at 37°C for 10 min. The preparations (approximately 100 μg protein/500 μl) were then transferred to plastic centrifuge tubes and incubated with continuous shaking (80 cycles/min) at 37°C for 15 min. The reaction was terminated by centrifugation (10,000*g* for 1 min) and the supernatants were stored at −80°C for the assay of eicosanoids (PGE_2, TxB_2, 6-keto-$PGF_{1\alpha}$, and LTB_4). The net production of eicosanoids was determined by subtracting the production of eicosanoids during 10 min of preincubation from the production of these compounds during 15 min of incubation.

Preparation of Enzyme Sources. Isolated glomeruli were washed twice with cold Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution and suspended in 2 ml of cold homogenate buffer (25 m*M* Tris-HCl, pH 7.5, containing 1 m*M* EDTA, 1 m*M* dithiothreitol and 100 kallidonogenase inactivator einheiten/ml of trazylol). Glomerular membrane preparations containing microsomal fractions were obtained by centrifugation at 100,000*g* for 60 min after 20 strokes of a 0.5-ml Teflon-glass homogenizer. The pellets were then resuspended in 250 μl of cold homogenate buffer and stored at −80°C as the enzyme sources of cyclooxygenase, PGE_2 isomerase, thromboxane and PGI_2 synthase, and 5-lipoxygenase.

Cyclooxygenase Mass. The mass of cyclooxygenase was determined as reported previously (3, 8). Glomerular membrane preparations were dissolved in sodium dodecyl sulfate sample buffer and heated for 2 min at 90°C. The preparations (5 μg of protein) were subjected to 13.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Laemmei (9) and electrophoretically transferred to a nitrocellulose membrane in 25 m*M* Tris buffer containing 190 m*M* glycine and 20% methanol. The nitrocellulose membrane was washed three times with 20 m*M* Tris-HCl, pH 7.5, containing 500 m*M* NaCl (Buffer A) to remove

methanol and then immersed in 20 mM Tris-HCl, pH 7.5, containing 3% gelatine, 500 mM NaCl, and 0.02% NaN_3 to saturate nonspecific protein binding sites on the membrane. After washing three times with 20 mM Tris-HCl, pH 7.5, containing 500 mM NaCl and 0.05% Tween 20 (Buffer B), the membrane was incubated for 60 min at room temperature with polyclonal cyclooxygenase antibody (1/1000 dilution) in Buffer B containing 1% gelatine. The nitrocellulose membrane was washed three times in Buffer B and further reacted for 60 min with alkaline phosphatase-conjugated sheep anti-rabbit IgG (1/9000 dilution) in Buffer B containing 1% gelatine. The sheet was then stained in 10 ml of 100 mM Tris-HCl buffer, pH 9.5, containing 5 mM $MgCl_2$, 100 mM NaCl, 0.75 mg of 5-bromo-4-chloro-3-indolyl phosphate, and 1.5 mg of nitroblue tetrazolium. The amounts of cyclooxygenase were measured by scanning the blots with a GS 300 densitometer (Hoefer Scientific Instruments, San Francisco, CA). Values of each band are in arbitrary units. All samples were run simultaneously to avoid interassay variation.

Cyclooxygenase and 5-Lipoxygenase Activities. The activities of cyclooxygenase and 5-lipoxygenase were measured as reported previously (3, 8). In brief, glomerular membrane preparations containing microsomal fractions in homogenate buffer were preincubated for 5 min at 37°C. The glomerular membrane preparations (approximately 200 μg protein/500 μl) were then incubated for 10 min at 37°C in the presence of exogenous 30 μM arachidonic acid. The reaction was terminated by adding 50 μl of 1 N HCl and the samples were placed on ice for 10 min. After neutralizing the samples with 50 μl of 1 N NaOH, the supernatants were stored at −80°C for the determination of cyclooxygenase and 5-lipoxygenase activity. The activities of cyclooxygenase and 5-lipoxygenase were determined from the production rates of PGE_2 and LTB_4, respectively, by glomerular membrane preparations.

PGE_2 Isomerase and PGI_2 and Thromboxane Synthase Activities. The activities of PGE_2 isomerase and PGI_2 and thromboxane synthase were determined according to the protocol of Fukuda *et al.* (7) with a slight modification. After preincubation for 5 min at 37°C, glomerular membrane preparations containing microsomal fractions (approximately 200 μg protein/500 μl) in homogenate buffer were reacted with 3.5 μM PGH_2 for 1 min at 37°C. Termination of the reaction was done by adding 50 μl of 25 mM $FeCl_2$, which transferred PGH_2 to 12-hydroxy-5,8,10-heptadecatrienoic acid immediately (7). The reaction mixtures were then left for 15 min at room temperature and the supernatants were stored at −80°C until measuring the activity of PGE_2 isomerase and PGI_2 and thromboxane synthase. The activities of PGE_2 isomerase, PGI_2 synthase, and thromboxane synthase were determined from the production rates of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2, respectively, by glomerular membrane preparations.

Determination of Eicosanoid Production and Statistical Analysis. The production of PGE_2, 6-keto-$PGF_{1\alpha}$, TxB_2, and LTB_4 was determined by assaying these eicosanoids in the supernatant buffer using commercially available immunoassay kits supplied by Cayman Chemical Co. The cross-reactivities of PGE_2, 6-keto-$PGF_{1\alpha}$, TxB_2, and LTB_4 antibodies were as follows: PGE_2 antibodies, <0.2% for 6-keto-$PGF_{1\alpha}$, TxB_2, and $PGF_{2\alpha}$; 6-keto-$PGF_{1\alpha}$ antibodies, 0.92% for PGE_2, <0.01% for TxB_2, and 2.1% for $PGF_{2\alpha}$; TxB_2 antibodies, <0.01% for PGE_2, 6-keto-$PGF_{1\alpha}$, and LTB_4 and 0.22% for $PGF_{2\alpha}$; LTB_4 antibodies, <0.1% for LTC_4, LTD_4, and LTE_4. Eicosanoid production was corrected for the protein content of glomerular preparations determined by the method of Lowry *et al.* (10) and expressed per mg protein per stated incubation time. Data reported represent means ± SE of values obtained from five separate glomerular preparations in each SOC or BUO group. Statistical analysis was performed by Student's *t* test.

Results

Table I shows data on the production rates (pg/mg protein/15 min of incubation) of PGE_2, 6-keto-$PGF_{1\alpha}$, TxB_2, and LTB_4 by isolated glomeruli from SOC (n = 5) and BUO (n = 5) rats. The production of PGE_2, a major eicosanoid synthesized by rat glomeruli, was significantly ($P < 0.025$) greater by 2.0 times in glomeruli isolated from rats with BUO than in those of SOC rats. Similarly, the synthesis of 6-keto-$PGF_{1\alpha}$, TxB_2, and LTB_4 was markedly ($P < 0.005$) elevated by 2.6, 2.0, and 4.6 times in glomeruli obtained from rats with BUO, respectively, when compared with glomeruli obtained from SOC rats. In the present study, we confirmed the previous observation that glomeruli from rats with BUO produced significantly greater amounts of eicosanoids than glomeruli from SOC rats (2, 3).

Figure 1 illustrates immunoblots for the determination of cyclooxygenase content in glomerular mem-

Table I. Eicosanoid Production by Glomeruli Isolated from SOC Rats and Rats with BUO[a]

	PGE_2	6-keto-$PGF_{1\alpha}$	TxB_2	LTB_4
SOC	1905 ± 256	107 ± 13	142 ± 6	128 ± 38
BUO	3783 ± 547[b]	276 ± 19[c]	285 ± 33[c]	592 ± 99[c]

[a] Isolated glomeruli were prepared from SOC rats and rats with BUO of 24 hr duration. Isolated glomeruli were incubated in 500 μl of Hanks' balanced salt solution for 15 min at 37°C and eicosanoids were measured in the supernatant buffer using specific immunoassays. Data are expressed per mg protein of each preparation and represent means (pg/mg protein/15 min) ± SE of values obtained from five separate preparations.
[b] $P < 0.025$ compared with the value of BUO versus SOC.
[c] $P < 0.005$ compared with the value of BUO versus SOC.

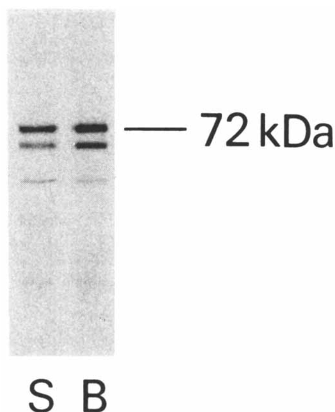

Figure 1. Immunoblots for cyclooxygenase in glomerular membrane preparations obtained from sham-operated control rats (S) and rats with bilateral ureteral obstruction (B). Glomerular membrane proteins (5 μg) were subjected to 13.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose sheet. The nitrocellulose sheet was incubated for 60 min with anticyclooxygenase antibody in immunoblotting buffer. After washing three times with wash buffer, the sheet was further incubated for 60 min with anti-rabbit IgG conjugated with alkaline phosphatase in immunoblotting buffer and stained as described in Materials and Methods.

Table II. Levels of Cyclooxygenase in Glomeruli Isolated from SOC Rats and Rats with BUO[a]

Cyclooxygenase	SOC	BUO
Immunoblot (relative mass)	0.98 ± 0.10	1.64 ± 0.12[b]
30 μM Arachidonate (relative activity)	1981 ± 162	3931 ± 528[c]

[a] Glomerular membrane preparations containing microsome were prepared from SOC rats and rats with BUO. The mass of cyclooxygenase was determined by scanning Western blots with a densitometer as described in Materials and Methods. Values are means (arbitrary units/5 μg protein) ± SE obtained from five separate glomerular membrane preparations. The activity of cyclooxygenase was determined in glomerular membrane preparations incubated with 30 μM arachidonic acid for 10 min at 37°C and measuring the formation of PGE_2 by specific immunoassay as described in Materials and Methods. Values are means ± SE of pg PGE_2 formed/mg protein/10 min obtained from five separate glomerular membrane preparations.
[b] $P < 0.005$ compared with the value of BUO versus SOC.
[c] $P < 0.01$ compared with the value of BUO versus SOC.

brane preparations from SOC rats and rats with BUO. The detailed characterization of anticyclooxygenase antibody used in the present study was described previously (3, 8). The anticyclooxygenase antibody specifically recognized cyclooxygenase proteins at 72 kDa in size in glomerular membrane preparations from SOC and BUO rats. The antibody, however, did not detect significant amounts of cyclooxygenase in cytosolic fractions of glomeruli obtained from the two groups of rats (data not shown).

Table II presents data on the mass and activity of cyclooxygenase in glomerular membrane preparations from SOC rats ($n = 5$) and rats with BUO ($n = 5$). The mass of cyclooxygenase was significantly ($P < 0.005$) greater by 1.7 times in rats with BUO than in SOC rats. Similarly, the activity of cyclooxygenase was markedly ($P < 0.01$) greater by 2.0 times in rats with BUO than in SOC rats when the activity of this enzyme was determined from the production rates of PGE_2 in the presence of exogenous 30 μM arachidonic acid added to glomerular membrane preparations. As with immunoblots, however, no activity of cyclooxygenase was detectable in cytosolic extracts of glomeruli obtained from SOC and BUO rats. Thus, bilateral ureteral ligation enhances the activity of cyclooxygenase which is related to *de novo* synthesis of this enzyme in rat glomerular membrane preparations.

Table III shows data on the activity of 5-lipoxygenase in glomerular membrane preparations from SOC ($n = 5$) and BUO ($n = 5$) rats. The activity of 5-lipoxygenase was determined by measuring the production rates of LTB_4 by glomeruli in the presence of exogenous 30 μM arachidonic acid. The activity of membrane-associated 5-lipoxygenase was greater by 3.1 times ($P < 0.025$) in rats with BUO than in SOC rats.

Table IV shows data on the activity of PGE_2 isomerase, PGI_2 synthase, and thromboxane synthase in

Table III. Activity of Membrane-Bound 5-Lipoxygenase in Glomeruli Isolated from SOC Rats and Rats with BUO[a]

	SOC	BUO
5-Lipoxygenase	286 ± 67	886 ± 179[b]

[a] Glomerular membrane preparations containing microsome were prepared from SOC rats and rats with BUO. The activity of membrane-bound 5-lipoxygenase was determined in glomerular membrane preparations incubated with 30 μM arachidonic acid for 10 min at 37°C and measuring the formation of LTB_4 by specific immunoassay as described in Materials and Methods. Values are means ± SE of pg LTB_4 formed/mg protein/10 min obtained from five separate glomerular membrane preparations.
[b] $P < 0.025$ compared with the value of BUO versus SOC.

Table IV. Activity of PGE_2 Isomerase, PGI_2 Synthase, and Thromboxane Synthase in Glomeruli Isolated from SOC Rats and Rats with BUO[a]

	SOC	BUO
PGE_2 isomerase	59,374 ± 6,972	125,358 ± 17,402[b]
PGI_2 synthase	1,219 ± 127	1,991 ± 236[c]
Thromboxane synthase	1,934 ± 429	3,563 ± 457[d]

[a] Glomerular membrane preparations containing microsome were prepared from SOC rats and rats with BUO. The activity of PGE_2 isomerase, PGI_2 synthase, or thromboxane synthase was determined in glomerular membrane preparations incubated with 3.5 μM PGH_2 for 1 min at 37°C and measuring the formation of PGE_2, 6-keto-$PGF_{1\alpha}$, or TxB_2 by specific immunoassays as described in Materials and Methods. Values are means ± SE of pg PGE_2, 6-keto-$PGF_{1\alpha}$, or TxB_2 formed/mg protein/min obtained from five separate glomerular membrane preparations.
[b] $P < 0.01$ compared with the value of BUO versus SOC.
[c] $P < 0.025$ compared with the value of BUO versus SOC.
[d] $P < 0.05$ compared with the value of BUO versus SOC.

glomerular membrane preparations from SOC ($n = 5$) and BUO ($n = 5$) rats. Each enzyme activity was determined from the production rates of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 when exogenous 3.5 μM PGH_2 was added to glomerular membrane preparations. The activity of PGE_2 isomerase was greater by 2.1 times ($P < 0.01$) in rats with BUO than in SOC rats. Similarly, the activity of PGI_2 and thromboxane synthase was increased by 1.6 ($P < 0.025$) and 1.8 ($P < 0.05$) times, respectively, in rats with BUO as compared with SOC rats. Moreover, the activity of PGE_2 isomerase was over 30 times greater than the activity of thromboxane synthase and over 48 times greater than the activity of PGI_2 synthase, which explains why glomeruli produce predominantly PGE_2 and, to a lesser extent, TxB_2 and 6-keto-$PGF_{1\alpha}$.

Discussion

We demonstrated previously that the activity of phosphatidylethanolamine (PE)-specific phospholipase A_2 (PLA_2) and cyclooxygenase was significantly greater in glomerular membranes from rats with bilateral ureteral obstruction of 24 hr duration than in glomerular membranes of sham-operated control rats (3). The increased activity of these enzymes in the membranes of rats with BUO was decreased to the values observed in the membranes of SOC rats by pretreating the rats with the angiotensin-converting enzyme inhibitor, enalaprilat, and the thromboxane synthase inhibitor, OKY-046, prior to obstruction (3). Blockade of the endogenous synthesis of both angiotensin II and thromboxane did not affect the activity of PE-specific PLA_2 and cyclooxygenase in glomerular membranes of SOC rats (3). Phosphatidylcholine-specific PLA_2 and phosphatidylinositol-4,5-biposphate-specific phospholipase C were not involved in the greater production of glomerular eicosanoids seen at 24 hr after the onset of BUO (3). Similarly, we found that the enhanced production of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 by glomeruli from rats with BUO of 24 hr duration was restored to the levels seen in SOC rats when endogenous angiotensin II was inhibited by enalaprilat given *in vivo* (2). Taken together, these observations indicate that angiotensin II-induced increases in the activity of PE-specific PLA_2 and cyclooxygenase may have a major role in the greater production of glomerular eicosanoids observed at 24 hr after the onset of BUO.

To further explore the mechanisms by which glomeruli of rats with BUO produced significantly greater amounts of eicosanoids than those of SOC rats, we examined the activity of several other enzymes involved in eicosanoid formation such as PGE_2 isomerase, PGI_2 and thromboxane synthases, and 5-lipoxygenase, and reexamined the activity and mass of cyclooxygenase in glomerular membrane preparations containing microsomal fractions obtained from SOC rats and rats with BUO of 24 hr duration. We confirmed our previous findings (2, 3) that glomeruli of rats with BUO had a significantly greater activity of cyclooxyenase involving *de novo* synthesis of this enzyme and produced substantially increased amounts of the vosodilatory prostaglandins, PGE_2 and prostacyclin (measured as the stable metabolite 6-keto-$PGF_{1\alpha}$), and the vasoconstrictor, thromboxane A_2 (measured as the stable metabolite TxB_2), when compared with glomeruli of SOC rats. Moreover, we found that BUO compared with SOC had significantly increased activities of PGE_2 isomerase, PGI_2 synthase, and thromboxane synthase in glomeruli. Thus, the present and previous (2, 3) studies indicate that BUO of 24 hr duration enhances the glomerular production of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 via the increased activity of the PE-specific PLA_2, the cyclooxygenase, and the respective synthases.

McNamara *et al.* (6) and Fukuda *et al.* (7) studied the activity of PGE_2 isomerase and PGI_2 and thromboxane synthases in the whole kidney of rats with unilateral ureteral obstruction. They found that by 72 hr after the onset of obstruction, the activity of PGI_2 and thromboxane synthases was significantly increased in the obstructed kidney of rats with unilateral ureteral obstruction when compared with the contralateral, untouched kidney of the same rats (6, 7). By contrast, the activity of PGE_2 isomerase in the obstructed kidney was comparable to that seen in the contralateral kidney (6, 7). Moreover, the activity of these three enzymes was not different between the contralateral kidney of rats with unilateral ureteral obstruction and the kidney of SOC rats (7). These findings are somewhat different from ours. The differences may be due to the diverse experimental models and preparations used: bilateral versus unilateral ureteral obstruction and glomeruli in our study versus whole kidneys in the studies of McNamara *et al.* (6) and Fukuda *et al.* (7).

The role of leukotrienes in rats with BUO of 24 hr duration has been examined in detail using MK886, an inhibitor of the translocation of the 5-lipoxygenase from its inactive cytosolic location to its membrane-bound active location (4). The *in vivo* inhibition of 5-lipoxygenase with MK886 ameliorated a marked fall in GFR and renal plasma flow occurring as a consequence of bilateral ureteral ligation to 50% of the values observed in sham-operated controls (4). These functional changes were paralleled by a reduction in the production of LTB_4 in glomeruli isolated from rats with BUO (4), suggesting that the vasoconstriction of the renal circulation in BUO rats is mediated in part by the increased production of vasoconstrictive leukotrienes such as LTB_4. Indeed, we demonstrated in the present study that the production of LTB_4 and the activity of membrane-bound 5-lipoxygenase in the active location of this enzyme were significantly increased in glomeruli of rats with BUO relative to glomeruli of SOC rats.

Bilateral ureteral ligation has been shown to cause an infiltration of leukocytes (predominantly macrophages and neutrophils) into glomeruli, which peaks at approximately 12–24 hr after the onset of obstruction (4, 11). Prior total body irradiation of rats with BUO almost completely abolished the leukocyte infiltration into the glomeruli (4) and significantly decreased the glomerular production of TxB_2 and LTB_4 when compared with rats with BUO that were not irradiated (4, 11). This decreased production of TxB_2 and LTB_4, however, was still increased significantly when compared with SOC rats (4, 11). These suggest that the increased production of glomerular TxB_2 and LTB_4 in the setting of BUO of 24 hr duration comes from the combined action of enhanced eicosanoid-forming enzymes derived from intrinsic glomerular cells and invading leukocytes of glomeruli.

The *in vitro* production of PGE_2, 6-keto-$PGF_{1\alpha}$, TxB_2, and LTB_4 was significantly greater in glomeruli isolated from rats with BUO than in glomeruli isolated from SOC rats. The activity of cyclooxygenase was significantly enhanced with the new enzyme synthesis in glomeruli isolated from rats with BUO. Also, the activity of other cyclooxygenase pathway enzymes—PGE_2 isomerase, PGI_2 synthase, and thromboxane synthase—was markedly greater in glomeruli isolated from rats with BUO. Moreover, glomeruli isolated from rats with BUO had a significantly greater activity of membrane-bound 5-lipoxygenase, which was the activated enzyme. Thus, we conclude that BUO of 24 hr duration increases the glomerular production of eicosanoids through the activation of both the cyclooxygenase and 5-lipoxygenase pathways.

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