Cytochrome P-450 Pathway in Renal Function of Normal Rats and Rats with Bilateral Ureteral Obstruction (43508)

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Abstract. **Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) are decreased and mean arterial pressure (MAP) and renal vascular resistance (RVR) are increased after unilateral release of bilateral ureteral obstruction (BUO) of 24 hr duration. An imbalance between vasoconstrictor and vasodilator substances may explain these hemodynamic changes. We examined the role of the cytochrome P-450 pathway in this setting. After unilateral release of BUO, GFR and ERPF (ml/min/kg body wt) were significantly lower in these rats than in sham-operated rats (SOR) 1.14 ± 0.09 vs 6.7 ± 0.5 and 3.09 ± 0.2 vs 23.5 ± 3.4, respectively). BUO rats had significantly higher MAP (mm Hg) and RVR (mm Hg/ml/min/kg body wt) than SOR (155 ± 5 vs 120 ± 1 and 29.1 ± 1.7 vs 3.2 ± 0.4, respectively). SOR given 3-methylcholanthrene and β-naphthoflavone to induce the cytochrome P-450 system had no significant changes in renal function, RVR, or MAP. SOR given ketoconazole to inhibit the cytochrome P-450 system had significantly lower GFR (4.8 ± 0.5) than temporal control rats without significant changes in ERPF (21.2 ± 4.6), MAP (127 ± 6), or RVR (4.2 ± 0.9). Rats with BUO given ketoconazole had lower but not significantly different GFR (0.84 ± .1) and ERPF (2.61 ± .4) than BUO controls. Values for MAP did not differ in BUO rats given ketoconazole versus BUO temporal controls. BUO rats given 3-methylcholanthrene and β-naphthoflavone had significantly higher GFR and ERPF (2.01 ± 0.24 and 6.66 ± 1.36, respectively) and significantly lower RVR (14.7 ± 3.9) than control rats with BUO; MAP was unchanged. Microsomal preparations from indomethacin-treated isolated kidneys obtained from BUO rats when compared with preparations obtained from SOR had significantly less activity of the P-450 cytochrome-dependent ω/ω-1 hydroxylase (103 ± 6 vs 130 ± 7 pmol hydroxyeicosatetraenoic acids produced per mg of protein/min, $P < 0.02$) and the P-450 cytochrome-dependent epoxygenase (11 ± 0.3 vs 30 ± 4 pmol lipoxyeicosatrienoic acids produced per mg of protein/min, $P < 0.04$). Indomethacin-treated microsomes prepared from kidneys of BUO rats converted significantly less ^{14}C-arachidonic acid through the P-450-dependent hydroxylases (13.5 ± 0.8 vs 17.0 ± 0.1% of ^{14}C-arachidonic acid converted to 19- and 20-hydroxyeicosatetraenoic acids, $P < 0.02$), and significantly less through the epoxygenases (1.4 ± 0.4 vs. 3.8 ± 0.5% of ^{14}C-arachidonic acid converted to epoxyeicosatrienoic acids). The data suggest that a metabolite(s) of the cytochrome *P*-450 pathway modifies renal function *in vivo* in such a way that inhibition or activation of the cytochrome *P*-450 system is reflected in decreases or increases in GFR and ERPF in BUO rats without affecting mean arterial pressure. We conclude that the cytochrome *P*-450 pathway plays an important role in renal hemodynamics of normal rats and rats with BUO of 24 hr duration.** [P.S.E.B.M. 1992, Vol 201]

Striking changes in glomerular and tubular function, renal vascular resistance, and mean arterial pressure occur in rats after unilateral release of bilateral ureteral obstruction (BUO) of 24 hr duration. Glomerular filtration rate (GFR) is about one third and effective renal plasma flow (ERPF) is about one fifth of normal (1). In addition, mean arterial blood pressure (MAP) and renal vascular resistance (RVR) are significantly increased in these rats (2). Some of the changes in renal hemodynamics may be due to an altered balance between vasodilatory and vasoconstrictive substances. Angiotensin II (3), antidiuretic hormone (4),

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endothelin (5), thromboxane A_2 (6), and leukotrienes (7) tend to decrease effective renal plasma flow and GFR, whereas prostaglandins (8), platelet-activating factor (9), and endothelium-derived relaxing factor (10) tend to maintain renal function. We have reported that blocking the effect (4, 5) or inhibiting (3, 6, 7) the synthesis of vasoconstrictors and/or administration of vasodilators (8–10) ameliorates, but does not restore to normal, renal function in rats with unilateral release of BUO of 24 hr duration (BUO rats). This suggests that increased activity of other vasoconstrictors and/or decreased activity of other vasodilators may play a role in the altered hemodynamics in BUO rats.

The present study was designed to examine the potential role of the cytochrome P-450 pathway in the hemodynamic alterations seen in BUO rats.

Materials and Methods

Animals. Studies were performed in 46 female Sprague-Dawley rats obtained from Sasco, Inc. (Omaha, NE) and weighing 173–294 g (mean, 220 ± 4 g). Rats were housed five or six to a cage and maintained in a 12:12-hr light:dark cycle at 21°C. They had free access to water and were fed a standard rat chow containing 22.8% protein (Ralston Purina, St. Louis, MO). All experiments were performed a minimum of seven days after arrival of the animals.

Induction of BUO or Sham Operation. These procedures were performed as described previously (9). Twenty-four rats underwent BUO and 22 rats were sham operated. For BUO, rats were placed under light ether anesthesia and both ureters were ligated at the junction of the lower one third and the upper two thirds through a small suprapubic abdominal incision. In sham-operated rats (SOR), both ureters of anesthetized rats were mobilized but not tied. After surgical procedures, rats were returned to their cages. SOR had free access to water. Both groups of rats were fasted for the next 24 hr and the functional studies described below were performed.

Clearance Studies and Mean Arterial Pressure Determinations. These procedures were performed in 33 rats as described previously (9). Briefly, 24 hr after either BUO or sham operation, the rats were lightly anesthetized with ether. A femoral vein catheter (PE-50) and a femoral artery catheter (PE-10) were inserted. A left ureteral catheter (PE-50) was placed above the ligature in BUO rats; a bladder catheter (Tygon) was inserted in SOR. No difference in renal function has been found when using urine obtained either from the bladder or from the ureter (11). The rats were secured in plastic holders and allowed to awake. After 2 hr, for recovery from anesthesia and surgery, a priming dose of inulin (Sigma Chemical Co., St. Louis, MO) and *para*-aminohippuric acid (PAH) (Merck, Sharp & Dohme, West Point, PA) was administered through the femoral vein catheter in 1 ml of normal saline over a 3-min period. The amounts given were designed to achieve plasma concentrations of 50–150 mg/dl for inulin and 1–2 mg/dl for PAH. The priming dose was followed by the infusion, through the femoral vein catheter at a rate of 40 μl/min, of a solution containing appropriate amounts of inulin and PAH to keep the plasma concentrations of these compounds constant. After 1 hr of equilibration, three 20-min urine collections were obtained with blood being drawn from the femoral arterial catheter at the midpoint of each urine collection. Blood samples were centrifuged immediately at 3000 rpm for 5 min; hematocrit was determined and plasma was separated for measurements of inulin and PAH. MAP was measured continuously through a transducer connected to the femoral arterial catheter (WECO, VT-1 model; Electronic Co, Millbrae, CA).

Inhibition of the Cytochrome P-450 System. Five SOR and eight BUO rats were given a single dose (25 mg/kg body wt) of ketoconazole (Sigma) 24 hr before sham operation or BUO. Ketoconazole was given to these two groups of rats under ether anesthesia by direct administration into the stomach through a small epigastric incision. Three other sham-operated rats and two BUO rats had surgery in which the gastric cavity was opened but no substance was administered. Pilot experiments demonstrated that higher doses (30–50 mg/kg/body wt) of ketoconazole induced hypotension and anuria in all rats, and death in BUO rats. Therefore, we progressively titrated the dose of ketoconazole until we were able to maintain the animals alive throughout the experiment, and this was achieved with a standard dose of 25 mg/kg body wt, an amount that did not affect mean arterial pressure in either SOR or BUO rats.

Activation of the Cytochrome P-450 System. Five SOR and four BUO rats were treated on each of 3 days before the surgical procedure with 80 mg/kg body wt of 3-methylcholanthrene (3MC) and 80 mg/kg body wt of β-naphthoflavone (βNF), according to the protocol described by Oyekan *et al.* (12). Both compounds were dissolved in 1 ml of peanut oil (Sigma) and given to the rats subcutaneously. Three other sham-operated rats and three BUO rats were given 1 ml of peanut oil in the same manner.

Determination of the P-450 Enzyme Activity in Renal Cortical Microsomes. Six SOR and seven rats with BUO of 24 hr duration were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg body wt), and both kidneys were harvested, decapsulated, and immersed in liquid nitrogen. Samples were stored at −80°C until assayed. Renal cortical microsomes were isolated as described previously (13). Briefly, 0.3 mg of microsomal protein was preincubated with 10 μM of indomethacin for 10 min at 37°C, then 0.4 μCi of ^{14}C-arachidonic acid (6.9 μM of ^{14}C-arachidonic acid) was added with an NADPH generating system composed of glucose-6-phosphate (0.4 mM), glucose-6-phosphate

dehydrogenase (1 unit), and NADP (1.0 mM) in potassium phosphate buffer (pH 7.6) to a final volume of 1 ml. The mixture was incubated for another 30 min at 37°C. The reaction was terminated by acidification with 1 M citric acid to pH 4.5. Arachidonic acid and its metabolites were extracted with ethylacetate and separated by high-pressure liquid chromatography. Reverse-phase high-performance liquid chromatography was performed on a C-18 Microsorb column (250 × 4.6 mm; Rainin Instrument Co., Inc., Woburn, MA) using a linear gradient of 1.25%/min from acetonitrile/water/acetic acid (50:50:0.1) to acetonitrile/acetic acid (100:0.1) at a flow rate of 1 ml/min. Radioactivity was monitored by a flow detector (Radiomatic Instrument and Chemical Co., Inc., Tampa, FL). Each extract was resuspended in 200 μl of methanol, and 75 μl of the solution were injected into the high-performance liquid chromatography column for separation. Compounds that eluted between 14.4 and 16.2 min comigrated with authentic standard of dihydroxyeicosatrienoic acids and 20-carboxy arachidonic acid, metabolites of epoxyeicosatrienoic acids (EET), and 20-hydroxyeicosatetraenoic acid (HETE), respectively. Compounds that eluted between 16.3 and 19.5 min comigrated with 19- and 20-HETE, products of ω-1- and ω-hydroxylases, respectively. Compounds that eluted between 24 and 26 min comigrated with authentic EET standards, products of epoxygenases. The percentage of conversion of arachidonic acid by ω- and ω-1-hydroxylase and epoxygenase was calculated and the enzyme activities were expressed as pmol/mg/min. The total P-450-arachidonic acid metabolism specific activity of the rat renal cortical microsomes was about 150 pmol/mg/min. Protein concentration was determined by the Lowry assay (14).

Experimental Groups of Rats for Renal Function Studies. Three groups of SOR and three groups of BUO rats were studied. Groups 1 through 3 consisted of SOR. Group 1 comprised six rats that served as temporal controls. Group 2 consisted of five SOR that received ketoconazole 24 hr before the surgical procedure. Group 3 consisted of five SOR that received 3MC and βNF 3 days before the surgical procedure. Groups 4 through 6 consisted of rats with BUO. Group 4 comprised five rats that served as temporal controls. Group 5 consisted of eight BUO rats that received ketoconazole 24 hr before study. Group 6 consisted of four BUO rats that received 3MC and βNF 3 days before the surgical procedure.

Analytic Determinations. Concentrations of inulin in plasma and urine were determined by the anthrone method of White and Samson (15). PAH in plasma and urine was measured by the method of Smith *et al.* (16).

Calculations and Statistics. Clearances of inulin and PAH were calculated according to a standard formula. In SOR, the values for GFR and ERPF were expressed per one kidney. RVR was calculated according to the formula: RVR = MAP/renal blood flow. Renal blood flow was calculated as ERPF/(1-[hematocrit/100]). Results are expressed as mean ± SE. Intergroup comparisons were performed by analysis of variance, with Bonferroni's correction. Differences were considered significant when $P < 0.05$.

Results

Enzymatic activity of the P-450 pathway of arachidonic acid metabolism in indomethacin-treated microsomal preparations of kidneys obtained from SOR and BUO rats is summarized in Table I. Conversion of ^{14}C-arachidonic acid to 19- and 20-HETE or EET was significantly lower in microsomal preparations from BUO rats than from SOR. The activity of the ω- and ω-1-hydroxylases and epoxygenase was also significantly lower in microsomal preparations obtained from BUO rats than in those obtained from SOR.

The effect on GFR and ERPF of maneuvers designed to inhibit or activate the cytochrome P-450 in SOR or BUO rats is depicted in Figure 1. The values for GFR and ERPF are the mean of three clearance periods. The results of renal function studies in the three SOR with gastric operation were similar and not significantly different from those obtained in the three SOR receiving peanut oil. Thus, the results of renal function studies in these six rats were pooled, and are expressed as a group. SOR given ketoconazole, an inhibitor of the cytochrome P-450 pathway (Group 2), had significantly lower GFR, but not ERPF, than temporal control rats (Group 1). Activation of the cytochrome P-450 pathway through the administration of 3MC and βNF did not significantly affect GFR or ERPF in SOR (Group 3). BUO rats (Groups 4–6) had GFR and ERPF values significantly lower than those of SOR (Groups 1–3). The results of renal function studies in the three BUO rats with gastric operation were similar and not significantly different from those obtained in the two BUO rats receiving peanut oil. Thus, the results of renal function studies in these five rats were pooled and are expressed as a group. Rats with BUO given ketoconazole prior to obstruction (Group 5) had lower GFR and ERPF values than BUO

Table I. Cytochrome P-450-Dependent Enzyme Activity in Microsomal Preparations from Kidneys of SOR and BUO rats[a]

	Conversion of ^{14}C-AA		Enzyme activity (pmol/mg/min)	
	19-/20-HETE	EET	ω/ω-1 Hydroxylase	Epoxygenase
SOR	17.0 ± 0.97	3.8 ± 0.5	130.4 ± 7.4	28.9 ± 3.6
BUO	13.5 ± 0.82	1.4 ± 0.04	103.2 ± 6.3	10.7 ± 0.3
P	<0.02	<0.004	<0.02	<0.004

[a] Results are mean ± SE. Abbreviations used in table: AA, arachidonic acid; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid.

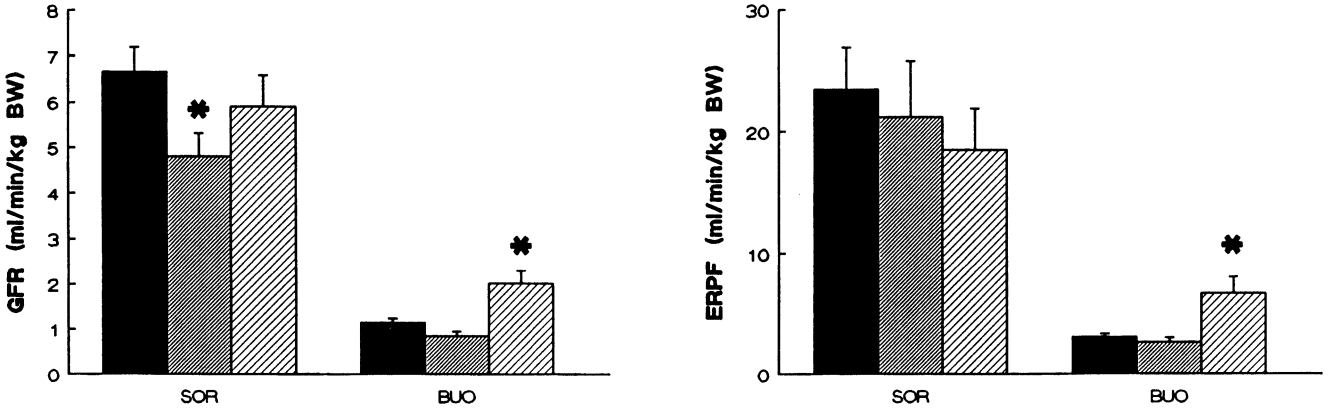

Figure 1. Effect of inhibition (administration of ketoconazole) or activation (administration of 3-methylcholanthrene + β-naphthoflavone) of the cytochrome P-450 pathway on GFR and ERPF of SOR or rats with unilateral release of BUO. *$P < 0.05$, compared with temporal controls. ■, temporal controls; ▨, +ketoconazole; ▨, +3MC-βNF.

rats that served as temporal controls (Group 4). However, that difference did not reach statistical significance. Rats with BUO given 3MC and βNF to activate the cytochrome P-450 pathway for a 3-day period prior to obstruction (Group 6) had significantly higher values for GFR and ERPF than BUO rats that served as temporal controls (Group 4).

The effect of manipulations of the cytochrome P-450 pathway on mean arterial pressure and renal vascular resistance is depicted in Figure 2. The three groups of BUO rats had significantly higher levels of MAP and RVR than the three groups of SOR. The different maneuvers designed to influence the activity of the cytochrome P-450 pathway did not significantly affect MAP in either SOR or BUO rats. SOR given ketoconazole (Group 2) or 3MC and βNF (Group 3) had slightly higher, but not significantly different, RVR values than temporal control rats (Group 1). BUO rats (Groups 4–6) had significantly higher RVR values than SOR (Groups 1–3). BUO rats given ketoconazole prior to obstruction (Group 5) had higher, but not significantly different, values for calculated RVR than BUO rats that served as temporal controls (Group 4). BUO rats given 3MC and βNF prior to obstruction (Group 6) had significantly lower RVR than BUO rats that served as temporal controls (Group 4).

Discussion

The results of this study indicate that a metabolite or metabolites of the cytochrome P-450 pathway play a role in the renal function of normal rats and, in particular, in the alterations of renal function seen in rats after unilateral release of BUO of 24 hr duration. However, the results obtained do not permit us to identify the specific substance or substances of the cytochrome P-450 pathway that modify renal function. It is possible that an unknown precursor leads to the formation of a vasoactive metabolite(s) with the ability to modify renal function *in vivo* in the awake rat. Several *in vitro* systems with relevance to renal physiology have been studied in which hormones or precursors of eicosanoid synthesis are metabolized by the cytochrome P-450. Using cyclo-oxygenase inhibition, several investigators (12, 17, 18) have examined the

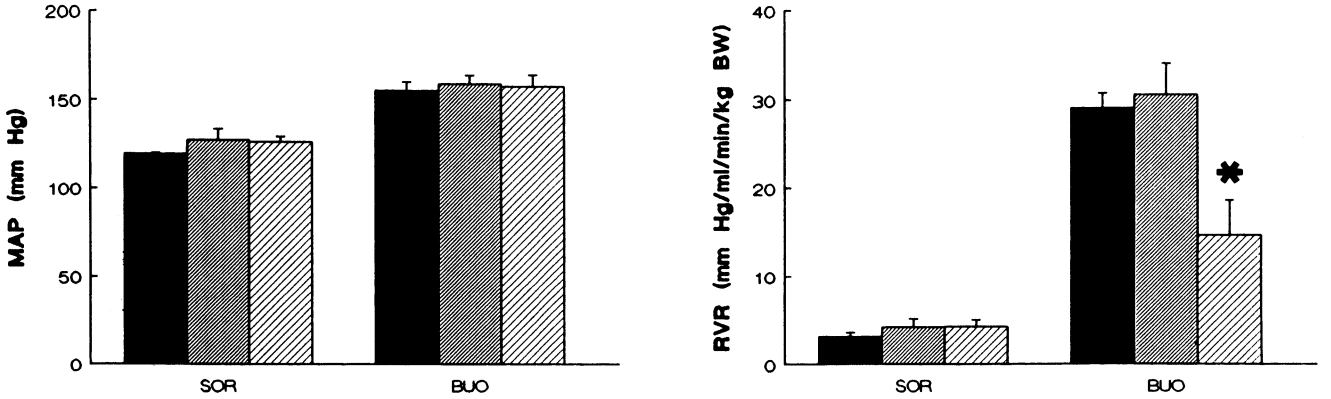

Figure 2. Effect of inhibition (administration of ketoconazole) or activation (administration of 3-methylcholanthrene + β-naphthoflavone) of the cytochrome P-450 pathway on MAP and RVR of SOR or rats after unilateral release of BUO. The three groups of rats with BUO had significantly higher levels of mean arterial pressure than the three groups of sham-operated rats. *$P < 0.05$, compared with temporal controls. ■, temporal controls; ▨, +ketoconazole; ▨, +3MC-βNF.

metabolism of arachidonic acid through the cytochrome P-450 pathway and have reported the *in vitro* production of a series of metabolites that affect renal function by inducing vasodilatation (EET) or changes in tubular sodium reabsorption (HETE). In the present studies, we found that conversion of labeled arachidonic acid through the P-450 metabolic pathway by microsomal preparations obtained from BUO rat kidneys was significantly less than in preparations obtained from SOR kidneys. This may suggest that in the setting of BUO of 24 hr duration, there is a small but significant reduction in the production of P-450 metabolites of arachidonic acid that may play a role in the alterations in renal function seen in this setting.

To examine the potential role of the P-450 system *in vivo*, we designed experiments in awake sham-operated and BUO rats to determine the effect on renal function of maneuvers that affect the P-450 cytochrome. We used compounds that either inhibit or induce the cytochrome P-450 pathway in a manner which is neither substrate nor organ specific. It is possible, therefore, that the substances metabolized by the cytochrome P-450 system that modify renal function can be synthesized within or outside of the kidney. Alternatively, it is also possible that substrate(s) metabolized by the P-450 system may not belong to the eicosanoid family of autacoid bioactive substances. We would like to propose that the effects on renal function of the nonspecific inhibition or induction of the cytochrome P-450 system are the result of the correspondent inhibition or activation of the synthesis of vasodilatory metabolites of arachidonic acid through this metabolic pathway. It should be pointed out, however, that ketoconazole given orally may bind irreversibly to the P-450 system in the liver upon first pass and may not affect the renal P-450 system.

In SOR, inhibition of the cytochrome P-450 by ketoconazole resulted in significantly lower GFR without significant changes in ERPF, MAP, or RVR. These effects suggest that a metabolic product or products of the cytochrome P-450 pathway participate in the maintenance of basal GFR, probably through an effect on single nephron GFR. On the other hand, induction of the cytochrome P-450 did not significantly change GFR in SOR.

Inhibition of the cytochrome P-450 system in BUO rats decreased GFR, although not significantly, when compared with temporal controls. Conversely, induction of the cytochrome P-450 system in BUO rats significantly increased GFR, but did not restore it to normal. Since the increase in GFR in BUO rats was accompanied by a significant decrease in RVR but not in MAP, the implication is that intrarenal hemodynamics are affected by the product(s) of this metabolic pathway. The present study does not allow us to determine whether this effect is mediated through changes in the ultrafiltration coefficient (K_f) or in arteriolar resistances.

Our results suggest a vasodilatory effect on the renal microcirculation of a product of the cytochrome P-450 system in normal rats and in BUO rats, but do not prove that arachidonic acid is the key substrate modified by the inducer or inhibitors of the P-450 cytochrome used in this study. These findings are in agreement with published reports of the *in vitro* synthesis of vasodilatory metabolites of arachidonic acid through the cytochrome P-450 pathway. Studies by Oyekan *et al.* (12) utilizing indomethacin-treated phenylephrine-preconstricted isolated perfused kidneys from rats unmasked a dose-dependent vasodilatory effect of arachidonic acid. This effect was enhanced by prior induction of the cytochrome P-450 system by pretreatment of the rats with 3MC and βNF. In those experiments, administration of arachidonic acid without blocking the cyclooxygenase pathway induced a dose-dependent increase in perfusion pressure. In addition, the same investigators demonstrated that depletion of the cytochrome P-450 system by pretreatment of the rats with stannous chloride or cobalt chloride (12), or inhibition of the cytochrome P-450 by treatment of rabbit kidneys *ex vivo* with clotrimazole or 7-ethoxyresorufin (17), significantly reduced the renal vasodilatory response to arachidonic acid. Experiments by Takahashi *et al.* (18) in isolated indomethacin-treated perfused kidney support a P-450-dependent vasodilatory effect of metabolites of arachidonic acid. In those experiments, administration of EET significantly decreased GFR and ERPF and that effect was opposed by pretreatment with a cyclooxygenase inhibitor. Our study confirms previous reports of *in vitro* observations that suggested a role for the P-450 system in the control of renal hemodynamics, and extends them to an *in vivo* model.

Modulation of renal function by metabolites of the cytochrome P-450 pathway of arachidonic acid is not surprising, since the kidney contains a number of cytochrome P-450 isoenzymes that are specific for arachidonic acid metabolism. Schwartzmann *et al.* (13) correlated renal microsomal P-450-dependent arachidonic acid metabolism with the level of cytochrome P-450 in the rabbit kidney. They found that the specific activity of the pathway was higher in the kidney than in the liver, with the highest activity being in the outer medulla.

In summary, the present studies demonstrate a vasodilatory role *in vivo* for the P-450 system in awake rats with unilateral release of bilateral ureteral obstruction of 24 hr duration. These effects appear to be mediated through regulation of intraglomerular hemodynamics. We conclude that a product or products of this metabolic pathway play a significant role in the hemodynamic alterations observed in urinary tract obstruction.

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1. Klahr S. Pathophysiology of obstructive nephropathy: A 1991 update. Semin Nephrol **11**:156–168, 1991.
2. Klahr S. Pathophysiology of obstructive nephropathy. In: Hatano M, Ed. Proceedings of the XIth International Society of Nephrology Meeting. Berlin: Springer-Verlag, pp78–93, 1991.
3. Purkerson ML, Klahr S. Prior inhibition of vasoconstrictors normalizes GFR in postobstructed kidneys. Kidney Int **35**:1306–1314, 1989.
4. Reyes AA, Robertson G, Klahr S. Role of vasopressin in rats with bilateral ureteral obstruction. Proc Soc Exp Biol Med **197**:49–55, 1991.
5. Reyes AA, Klahr S. Renal function after release of ureteral obstruction: Role of endothelin and the renal artery endothelium. Kidney Int **42**:632–638, 1992.
6. Klahr S, Harris KPG, Purkerson ML. Effect of obstruction on renal functions. Pediatr Nephrol **2**:34–42, 1988.
7. Reyes AA, Lefkowith J, Pippin J, Klahr S. Role of the 5-lipoxygenase pathway in obstructive nephropathy. Kidney Int **41**:100–106, 1992.
8. Klahr S, Buerkert J, Morrison A. Urinary tract obstruction. In: Brenner BM, Rector FC Jr, Eds. The Kidney. Philadelphia: WB Saunders, pp1443–1490, 1986.
9. Reyes AA, Klahr S. Role of platelet-activating factor in renal function in normal rats and rats with bilateral ureteral obstruction. Proc Soc Exp Biol Med **198**:572–578, 1991.
10. Reyes AA, Martin D, Settle S, Klahr S. EDRF role in renal function and blood pressure of normal rats and rats with obstructive nephropathy. Kidney Int **41**:403–413, 1992.
11. Reyes AA, Klahr S. Bladder contributes to eicosanoids excreted in urine. Am J Physiol **259**:F851–F861, 1990.
12. Oyekan AO, McGiff JC, Quilley J. Cytochrome p-450-dependent vasodilatation of the rat kidney by arachidonic acid. Am J Physiol **261**:H714–719, 1991.
13. Schwartzmann ML, Abraham NG, Carroll MA, Levere RD, McGiff JC. Regulation of arachidonic acid metabolism by cytochrome p-450 in rabbit kidney. Biochem J **238**:283–290, 1986.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem **193**:265–275, 1951.
15. White RP, Samson FE. Determination of inulin in plasma and urine by use of anthrone method. J Lab Clin Med **43**:475–478, 1954.
16. Smith HW, Finkelstein N, Aliminosa L, Crawford B, Graber M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J Clin Invest **24**:388–404, 1945.
17. Oyekan AO, McGiff JC, Quilley J. Cytochrome p-450-dependent vasodilator responses to arachidonic acid in the isolated, perfused kidney of the rat. Circ Res **68**:958–965, 1991.
18. Takahashi K, Capdevilla J, Karara A, Falck JR, Jacobson HR, Badr KF. Cytochrome p-450 arachidonate metabolites in rat kidney: Characterization and hemodynamic responses. Am J Physiol **258**:F781–F789, 1990.