Obstructive Nephropathy in the Rat

POSSIBLE ROLES FOR THE RENIN-ANGIOTENSIN SYSTEM, PROSTAGLANDINS, AND THROMBOXANES IN POSTOBSTRUCTIVE RENAL FUNCTION

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ABSTRACT Relief of unilateral ureteral obstruction (UUO) of 24 h duration in rats is followed by severe renal vasoconstriction in the postobstructive kidney (POK). The present study examined possible roles of renal prostaglandins (PG) and thromboxanes (TX), as well as the renin-angiotensin system, in this vasoconstriction.

Administration of the cyclooxygenase inhibitor indomethacin, which blocks both PG and TX production, failed to improve POK hemodynamics in UUO rats. To explore the possible role of the TX compounds, which include the potent vasoconstrictor thromboxane A2 (TXA2), UUO rats were infused with imidazole, an agent that blocks synthesis of TX, but not of PG. Imidazole led to two- to threefold increases in the clearance of both inulin and ρ-aminohippuric acid by the POK. This effect of imidazole was abolished by indomethacin, suggesting that the amelioration of POK vasoconstriction by imidazole was a result of inhibition of vasoconstrictor TX synthesis (e.g. TXA2), with PG vasodilators (e.g. PGE2 or PGI2) still active. Urea, infused in a solution whose osmolality and volume were identical to the imidazole infusion, failed to improve hemodynamics in the POK, making it unlikely that nonspecific effects of volume expansion or osmotic diuresis mediated the beneficial effect of imidazole.

Further studies examined the possible role of the renin-angiotensin systems in the vasoconstriction of the POK. UUO rats infused with the angiotensin II antagonist, Saralasin, exhibited no significant improvement in POK function, a finding that might be at least partly attributable to agonist/vasoconstrictor properties of Saralasin. In other experiments, treatment of UUO rats with the angiotensin-converting enzyme blocker SQ 14225 (Captopril), in order to inhibit angiotensin II formation, led to at least twofold increases in the clearance of both inulin and ρ-aminohippuric acid in the POK. It is unlikely that Captopril exerted this beneficial effect by potentiating the vasodilator kinins, because the effect was not diminished by administration of either carboxypeptidase B (which destroys the kinins) or Trasylol (which blocks kinin synthesis).

Thus, these results suggest that both angiotensin II, as well as metabolites of the PG-TX system, may be important determinants of postobstructive renal hemodynamics in the rat.

INTRODUCTION

Chronic obstruction of the urinary tract is associated with a severe reduction in renal blood flow and glomerular filtration rate, even after the obstruction is relieved (1, 2). Although structural alterations are undoubtedly important in increasing renal vascular resistance when hydronephrotic destruction of the kidney parenchyma has occurred, both blood flow and glomerular filtration have been shown to be severely decreased after the release of short periods of ureteral ligation, at a time when morphological changes are minimal or absent. This increase in renal vascular resistance can be somewhat ameliorated by extracellular volume expansion (3) or the infusion of renal vasodilators (4), suggesting that functional changes are at least partially responsible for the observed decrease in renal blood flow and glomerular filtration rate.

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Dr. Harris is an Established Investigator of the American Heart Association. He was a Research Associate of the Veterans Administration during the performance of part of these studies.

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In this study, we explored the potential contribution of renin and angiotensin as well as renal prostaglandins and thromboxanes to postobstructive renal function by treating rats with pharmacological agents which are capable of modifying these two hormonal systems. The renin-angiotensin system was investigated because renal renin production is known to be increased after acute ureteral obstruction (5) and because vasoconstriction in the postobstructive kidney (POK)[1] is most severe in the outer cortex (1, 2)—an area rich in renin-containing glomeruli (6). The potential role of renal prostaglandins and thromboxanes was explored because the production of prostaglandin E2 (PGE2) and thromboxane A2 (TXA2) by the in vitro perfused hydronephrotic rabbit kidney have been shown to be increased (7–9). The rat was chosen for this study, however, because this experimental animal demonstrates particularly severe vasoconstriction after the release of only 24 h of unilateral ureteral obstruction (UUO) (2).

Knowledge of prostaglandin biochemistry is expanding rapidly, and it is becoming clear that important differences exist in the effects that various prostaglandins produce in different organs and species. Therefore, some of the features of prostaglandin metabolism that are of importance to the present study are shown in Fig. 1. The rate-limiting step in prostaglandin biosynthesis appears to be the release of arachidonic acid from membrane-bound phospholipids. The phospholipase responsible for this release of arachidonic acid is stimulated by a variety of conditions including: injury, anoxia, and the action of several peptide hormones such as angiotensin II (AII) and bradykinin. Arachidonic acid is converted by the enzyme cyclooxygenase to the cyclic endoperoxides PGG2 and PGH2. Cyclooxygenase can be inhibited by a variety of nonsteroidal anti-inflammatory agents such as aspirin, indomethacin, and meclofenamate, thereby blocking further prostaglandin and thromboxane production. The cyclic endoperoxides can be converted into a variety of prostaglandins (PGE2, PGI2 or prostacycline, PGF2a, PGD2, etc.) or into the potent vasoconstrictor TXA2. TXA2, which has a very short half-life, decomposes spontaneously into TXB2, which is stable but biologically inactive. The enzyme which converts the cyclic endoperoxides into TXA2, thromboxane synthetase, has been detected in the glomeruli of normal rats (10). This enzyme has been shown to be inhibited by the pharmacological agent imidazole in microsomes isolated from platelets (11) and hydronephrotic rabbit kidneys (8, 9).

[1] *Abbreviations used in this paper*: AI, AII, angiotensins I and II; CIn, CPAH, COsm, CNa, clearance rates of inulin, ρ-aminohippuric acid, osmoles, and sodium, respectively; CK, contralateral kidney; PG-, prostaglandins such as: PGG2, PGH2, PGE2, PGI2; POK, previously obstructed kidney; TX, thromboxanes such as TXA2, TXB2; UUO, unilateral ureteral obstruction.

METHODS

133 male Sprague-Dawley rats weighing 250–350 g were studied. The animals were divided into 12 groups as shown in Table I. All animals, except the 11 rats in group VI, were subjected to ureteral obstruction 25 h before beginning clearance studies, by anesthetizing them with ether, making a small suprapubic incision, and ligating the left ureter between the bladder and the left spermatic cord. The incision was sutured and the animals were allowed to recover in separate cages. Solid food was withheld, but the rats received 50 ml of a 5% sucrose solution containing 3 meq each of NaCl and KCl. They also received tap water ad lib.

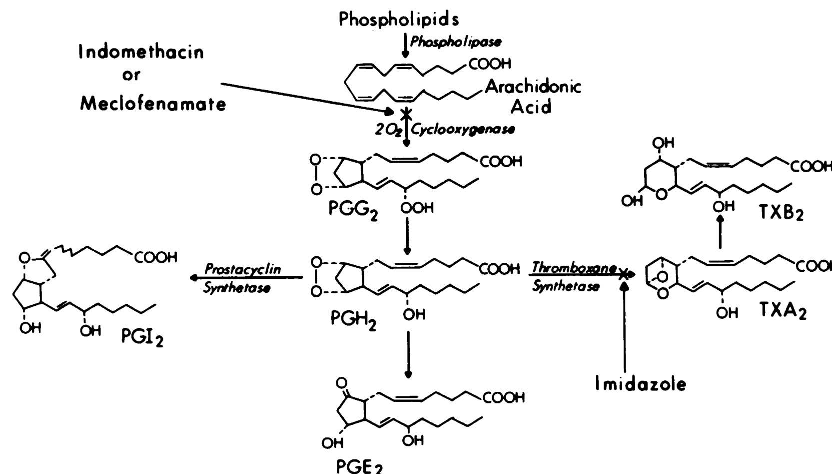

FIGURE 1 Metabolic pathways of prostaglandin and thromboxane synthesis in the kidney. The cyclooxygenase blockers, indomethacin and meclofenamate, inhibit the conversion of arachidonic acid to PGG2, thus inhibiting subsequent synthesis of both prostaglandins and thromboxanes. Imidazole, by selectively blocking thromboxane synthesis, inhibits synthesis of the vasoconstrictor thromboxane A2 (TXA2) without inhibiting synthesis of the vasodilators PGE2 and PGI2.

TABLE I
Experimental Groups, Number of Rats (n), Experimental Models, and Drug Treatments during Each Clearance Period

Group	n	Model	Treatment Period 1	Treatment Period 2	Treatment Period 3
I	10	UUO*	None	Indo	x‡
II	22	UUO	None	Imid	x
III	10	UUO	None	Urea	x
IV	11	UUO	Imid	Imid-Indo	x
V	11	UUO	Imid	Imid-Buff	x
VI	11	Normal	Imid	x	x
VII	7	UUO	Sarl	x	x
VIII	6	UUO	Cap-Pre	x	x
IX	6	UUO	Veh-Pre	x	x
X	20	UUO	None	Cap	x
XI	8	UUO	None	Cap	Cap-Cp B
XII	11	UUO	None	Cap	Cap-Tras

* *Abbreviations used in this table*: Normal, otherwise healthy, unoperated rats; None, no drugs were infused during these periods; Indo, indomethacin was given intravenously 30 min before this period; Imid, imidazole was infused intraarterially during these periods; Urea, was infused intraarterially during this period; Imid-Indo, imidazole infusion was resumed after indomethacin treatment; Imid-Buff, imidazole infusion was resumed after these rats had been infused with the buffer used to dissolve the indomethacin; Sarl, Saralasin; Cap-Pre, these rats were treated with Captopril before ureteral obstruction was produced; Veh-Pre, these rats were treated with the vehicle used to dissolve Captopril before ureteral obstruction was produced; Cap, these animals received Captopril just before these periods; Cap-Cp B, the rats received carboxypeptidase B 1 h after Cap; Cap-Tras, these rats received Trasylol beginning 1 h after Cap.

‡ There were no studies performed during these times.

On the day of study, all animals were anesthetized with 100 mg/kg Inactin i.p., placed on a thermoregulated table, and a short segment of polyethylene (PE)-240 catheter was inserted into the trachea to assist in spontaneous respiration. A PE-50 catheter was placed into the left femoral artery to permit periodic sampling of arterial blood and to measure arterial blood pressure. Blood pressure was monitored with an electronic pressure transducer (model P23Db, Gould Inc., Measurement Systems Div., Oxnard, Calif.) connected to a direct writing recorder (model 7754 B, Hewlett Packard Co., Palo Alto, Calif.). A PE-50 catheter was also inserted into the left femoral vein to infuse a priming dose of [*carboxyl*-14C]-inulin and [*glycyl*-^{3}H]p-aminohippuric acid (New England Nuclear, Boston, Mass.) followed by a continuous infusion of these isotopes in a solution of Ringer's lactate at a rate of 0.02 ml/min. In 65 rats a PE-10 catheter was also inserted through the left carotid or right femoral artery and was positioned in the aorta just above the renal arteries to infuse a solution of imidazole or Ringer's lactate. PE-50 catheters were also inserted into the left ureter above the ligature and into the bladder to collect urine from both the POK and the contralateral kidney (CK) for calculation of urine flow rate and the determination of inulin (CIn) and p-aminohippuric acid (CPAH) clearance. During this period of surgical preparation, the rats in groups I–VI received 1% of their body weight of a solution of Ringer's lactate. Because the type of surgical preparation used in these studies may lead to loss of plasma protein and a contraction of the plasma volume (12), to study the role of the renin-angiotensin system in the pathogenesis of ureteral obstruction, the rats in groups VII–XII received 1% of their body weight of a solution containing bovine serum albumin (7 g/dl) in Krebs-Henseleit buffer over a 1-h period to insure euvolemia during the subsequent clearance studies (13).

Urine collections were begun ~60 min after inserting the left ureteral catheter and continued for 2–3 h thereafter. Urine was collected in weighed containers for clearance periods lasting 20–30 min. Arterial blood was sampled at the midpoint of each urine collection and arterial blood pressure was measured throughout the experiment.

Prostaglandin and thromboxane studies. 75 rats in six experimental groups (groups I–VI, Table I) were used to study the effects of altering prostaglandin and thromboxane metabolism on postobstructive renal function. These rats were treated with several pharmacologic agents. 21 rats (groups I and IV) were treated with indomethacin to block both prostaglandin and thromboxane synthesis. The indomethacin was prepared by dissolving 0.179 g of indomethacin (Sigma Chemical Co., St. Louis, Mo.) and 0.106 g Na_2CO_3 in 10 ml of distilled water. This solution was diluted with pH 7.4 0.3 M phosphate buffer to a final indomethacin concentration of 3 mg/ml. The rats were infused intravenously with the solution over a 30-min period to administer a dosage of 10 mg/kg.

55 rats (groups II, IV–VI) were infused with imidazole to block the production of TXA2 (11). The imidazole (0.6 M) was prepared fresh daily by dissolving 0.408 g of imidazole (Sigma Chemical Co.) in 10 ml of a 0.9% solution of NaCl and adjusting the initially alkaline pH to a final value of 7.5 with HCl. This solution was infused intraarterially through a catheter positioned in the aorta above the renal arteries, at a rate of 0.075 ml/kg per min.

To assess the effects of the osmotic and volume load imposed by the imidazole infusion, 10 animals (group III) received an intraarterial infusion of 0.6 M urea solution. This urea solution was prepared by dissolving 0.360 g of urea (Sigma Chemical Co.) in 10 ml of a 0.9% solution of NaCl and then adjusting the pH to 7.5 with NaOH. The position of the catheter and the rate of administration were identical to that described for imidazole above.

11 rats (group V) were infused with the same buffer solution which was used to administer the indomethacin under identical conditions. The imidazole infusion in groups IV and V was temporarily discontinued and was replaced by an infusion of Ringer's lactate solution (0.075 ml/kg per min) during the period (2–2½ h after release) in which either indomethacin (group IV) or its buffer solution (group V) was infused; the imidazole infusion was then resumed.

Finally, group VI of normal healthy rats, not previously subjected to ureteral obstruction, was infused with imidazole to determine if this drug is a vasodilator in normal kidneys as well as in the kidneys of rats after the release of ureteral obstruction.

AII studies. 58 rats in six experimental groups (groups VII–XII, Table I) were used to determine whether blockade of AII activity (group VII) or its production (groups VIII–XII) can improve renal function after the release of 24 h of UUO.

Seven UUO rats (group VII) were given an intravenous infusion of the AII antagonist, [1-sarcosine, 8-alanine] AII (Saralasin) at rates of 2, 5, or 10 μg/kg per min after release of UUO. In two of these seven rats, the antagonist was infused both before and after release of UUO.

45 rats (groups VIII, X–XII) were subjected to blockade of

angiotensin-converting enzyme with Captopril (SQ 14225, kindly supplied by Dr. Z. P. Horovitz, The Squibb Institute for Medical Research, Princeton, N. J.). 50 mg/ml Captopril in 0.9% NaCl, was injected intravenously over a 5-min period to administer a 10-mg/kg dose. This dosage is sufficient to block at least 50% of converting enzyme activity in the rat in vivo for 24 h.[2] This dose of Captopril was given to the six rats in group VIII under ether anesthesia 30 min before the ureter was ligated. As a control group for the rats in group VIII, six rats (group IX), matched for weight with group VIII rats, received a similar volume of 0.9% saline solution under identical conditions. 39 rats (groups X–XII) received Captopril 2 h after the ureter was released. Clearance studies in these rats were initiated before Captopril treatment—hours 1–2 after releasing the ureter—so that the values from this initial period could serve as an untreated control with which values from subsequent treatment periods could be compared.

The angiotensin-converting enzyme not only converts AI to AII, but also inactivates vasodilator kinins (bradykinin, kallidin, and met-lys-bradykinin) (14). Therefore, to determine if the beneficial effects of Captopril treatment observed in group X were the result of decreased AII production or, conversely, to decreased kinin destruction, two additional groups of rats (groups XI and XII) were studied. In group XI, the destruction of bradykinin and other kinins was increased by infusing carboxypeptidase B (Worthington Biochemical Corp., Freehold, N. J.), prepared by dissolving 10 mg in 1.0 ml of a 0.9% saline solution. Because this enzyme is rapidly cleared by the kidney (15), a 3-mg/kg dose was injected at 3 and 3½ h after the release of the ureter while clearance studies were performed as shown in Table I.

In 11 rats (group XII) the production of bradykinin and other kinins was suppressed by infusing the kallikrein inhibitor (16, 17), Trasylol (registered trademark Bayer A. G., kindly supplied by Professor Dr. Gurt L. Haberland, Farbenfabriken, Bayer A. G., Wuppertal-Elberfeld, Germany). The Trasylol was prepared by dissolving 10.4 mg in 1.0 ml of a 0.9% solution of NaCl (69,000 KIU/ml). This solution was infused for 1 h at a rate of 0.033 ml/min beginning 3 h after the ureter was released as shown in Table I.

Osmolality and the concentration of sodium of the urine and plasma were measured in 10 of the 22 rats in group II and the 10 rats in group III. Osmolality was measured by a vapor pressure osmometer (model 5100, Wescor Inc., Logan, Utah). Sodium was measured by a lithium internal standard flame photometer (model 443, Instrumentation Laboratory, Inc., Lexington, Mass.). Plasma calcium was measured in seven rats in group II and seven rats in group IV by fluorometric titration with a Calcett Automatic Calcium Titrator (Precision Systems, Inc., Sudbury, Mass.). Imidazole concentrations were measured in deproteinized plasma from 11 rats in group II and six rats from group IV by modifying the procedure of Koessler and Hanke (18). The color developed by the reaction between imidazole and *p*-phenyldiazonium sulfonate was read at 520 nm at 15, 20, and 25 min after combining the reagents.

Calculations. Statistics were calculated according to standard methods. To obviate the effect that seasonal differences appear to exert on basal postrelease renal function (19), the animals were used as their own controls wherever possible, and the data were analyzed by the paired Student's *t* test. Where data from different groups are compared, the animals were matched by weight and an unpaired *t* test was utilized. A probability value <0.05 was regarded as significant. Values are presented as means±standard error of the mean.

[2] Dr. Z. P. Horovitz. Personal communication.

RESULTS

Previous studies in our laboratory have demonstrated that, after relief of 24 h of UUO in rats, the POK is intensely vasoconstricted (2). The present experiments were designed to examine the mechanisms of this vasoconstriction. It has been shown that the in vitro perfused hydronephrotic rabbit kidney exhibits increased production of both the vasoconstrictor TXA2 (8, 9) and PGE2 (7), which is a vasodilator in the rabbit kidney (20). Accordingly, we studied the role of prostaglandin and thromboxane metabolism in vivo in rats previously subjected to 24 h of UUO. The results of these studies are presented in Tables II and III and in Figs. 2 and 3.

The initial step in clarifying the roles of prostaglandins and thromboxanes after UUO was to suppress the production of both by blocking the enzyme cyclooxygenase (Fig. 1) with indomethacin. Because this enzyme converts arachadonic acid to the cyclic endoperoxides (PGG2 and PGH2), which are the precursors of both prostaglandins and thromboxanes, indomethacin treatment should allow examination of postobstructive renal function under circumstances in which the contribution of all of the metabolites of arachidonic acid have been markedly reduced or abolished. As shown in Table II, the group I data demonstrate that indomethacin treatment improves neither CIn nor CPAH by the POK. CPAH by the CK was slightly decreased.

These data are subject to several possible interpretations, one being that reduced production of both vasodilators (e.g. PGE2 or PGI2) as well as vasoconstrictors (e.g. TXA2) might have no net beneficial effect on the kidney. To test this hypothesis, group II rats were treated with imidazole, an agent which should decrease the production of thromboxanes, but not the production of prostaglandins (Fig. 1). As shown by the data in Table II and Fig. 2, imidazole treatment results in a significant increase in the values for both CIn and CPAH in the POK, suggesting that specific blockade of thromboxane production is quite beneficial to the POK.

However, as CIn (but not CPAH) also increased in the CK, and as function in the POK is known to be increased by volume expansion (3), it is possible that some nonspecific effect of an intraarterial infusion of a solution of high osmolality (850–900 mosmol/kg H_2O), rather than a metabolic effect of imidazole, might have been responsible for this improvement in renal function. To evaluate this possibility, a solution of urea in saline that was of similar volume, osmolality, and pH was infused in the rats in group III. The data from these rats were compared with those of 10 of the group II rats, as shown in Table III.

The data in Table III demonstrate the contrast between imidazole and urea with regard to their effects on renal hemodynamics and on the excretion of water and electrolytes. Because the rats in group III appeared

TABLE II

Effect of Various Treatments Designed to Alter Renal Prostaglandin and Thromboxane Metabolism after the Release of 24 h of Ureteral Obstruction

Group	POK				CK			
	Hours after release of the ureter				Hours after release of the ureter			
h..........1–4	1	2	3	4	1	2	3	4
I $n = 10$*	<----No treatment----><--Indo--><------ Post-Indo ------>				<---No treatment---><--Indo--><----- Post-Indo ----->			
	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH
	0.50‡ ±0.07	2.62 ±0.53	0.77 ±0.20	2.51 ±0.56	4.19 ±0.41	9.37 ±1.09	3.40 ±0.26	6.10 ±0.49
P			NS	NS			NS	<0.02
II $n = 22$	<----No treatment----><---------- Imidazole ------>				<---No treatment---><---------- Imidazole ----->			
	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH
	0.82 ±0.13	3.53 ±0.50	2.19 ±0.24	9.82 ±1.44	4.94 ±0.26	13.58 ±1.03	5.97 ±0.32	13.03 ±1.18
P			<0.001	<0.001			<0.001	NS
IV $n = 11$	<------ Imidazole ------><--Indo--><------ Imidazole ------>				<----- Imidazole -----><--Indo--><----- Imidazole ----->			
	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH
	2.47 ±0.62	10.81 ±2.13	0.83 ±0.47	3.22 ±0.73	5.92 ±0.47	14.74 ±1.04	4.70 ±0.47	11.62 ±0.93
P			<0.02	<0.01			NS	<0.02
V $n = 11$	<------ Imidazole ------><-Buffer-><------ Imidazole ------>				<----- Imidazole -----><-Buffer-><----- Imidazole ----->			
	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH
	1.71 ±0.25	6.28 ±0.71	1.78 ±0.27	6.28 ±0.91	5.28 ±0.24	11.51 ±1.06	5.52 ±0.13	11.15 ±0.68
P			NS	NS			NS	NS
VI§ $n = 11$					<---No treatment---><---------- Imidazole ----->			
					CIn	CPAH	CIn	CPAH
					4.48 ±0.26	10.44 ±0.97	4.85 ±0.31	10.63 ±1.53
P							NS	NS

* n, number of rats; NS, $P > 0.05$.
‡ Values are ml/kg per min, means±SE.
§ Group VI rats were not subjected to ureteral ligation.

to be slightly more diuretic and natriuretic during the control period (Pre) before being treated with urea, than were the 10 rats from group II, excretory data from the two groups are not compared with each other; rather, the data within each group before treatment (Pre) are compared with the data from that group after treatment (Post).

Although the values for both CIn and CPAH of the POK of the two groups shown in Table III were not significantly different during the control (Pre) period, imidazole treatment improved both parameters so that after treatment (Post) the values for both CIn and CPAH in the group II rats were considerably greater ($P < 0.001$ for each) than the values in the group III urea-treated rats. Therefore, these data suggest that the effects of imidazole on renal hemodynamics are not related nonspecifically to the volume or osmolality of the infusion, but to some pharmacological effect of the imidazole.

In addition, the data of Table III indicate that imidazole, but not urea, may lessen the concentrating defect that is characteristic of the POK (2), in that both U/P In and U/P Osm increased the ratio of urine to plasma

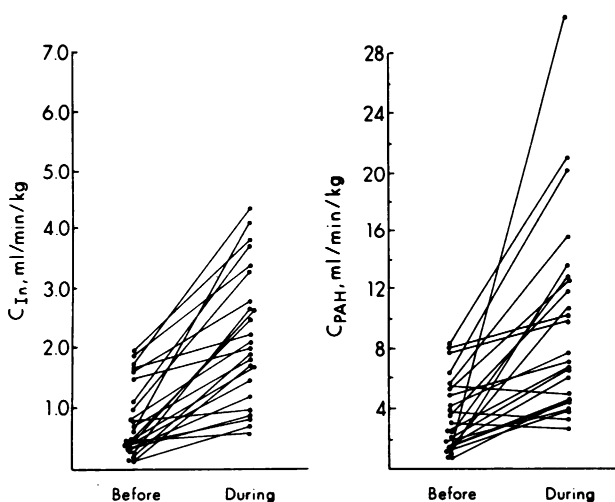

FIGURE 2 CIn and CPAH in the POK before and during inhibition of thromboxane synthetase with imidazole.

osmolality and inulin concentration significantly. Whether this improvement resulted from some direct effect of imidazole on the concentrating mechanism per se, or derived secondarily from the improvement in renal hemodynamics, cannot be determined from these data, however.

Although imidazole increased the absolute rate of sodium excretion, this appeared to result primarily from the increase in filtered load, since the fraction of filtered sodium excreted in the urine did not increase. The value for fractional osmolar clearance by the POK decreased slightly, but not significantly, in both groups of rats. This suggests that any effect of either agent cannot be explained by an osmotic diuresis, a conclusion further supported by the observation that neither agent produced a significant increase in plasma osmolality. Finally, imidazole produced a significant increase in blood pressure whereas urea did not. This increase in blood pressure after imidazole treatment also occurred in all other rats in group II.

Comparison of the data in groups I and II suggests that inhibition of thromboxane production (both groups) is not beneficial unless prostaglandin production continues unimpeded (imidazole alone). To test this hypothesis further, the rats in group IV were treated with imidazole before and after being infused with indomethacin. The results are shown in Table II and Fig. 3. Inspection of Table II reveals several interesting findings. First, as would be expected from the results in group II, it is clear that the function of the POK during the first imidazole period, before the infusion of indomethacin, is considerably better than the function observed in rats in groups I–III before any type of treatment was initiated. Second, it is clear that function by the POK during the second imidazole treatment period, which followed the infusion of indomethacin, deteriorated substantially and significantly as compared to values observed before indomethacin was given.

Because imidazole is an agent with many pharmacological effects, and because we found in preliminary studies[3] that prolongation of the imidazole infusion for 4 h or more resulted in depressed renal function, hypotension, hypocalcemia, and death, it is possible that the deterioration of renal function in the POK of the rats in group IV during the second imidazole period might have been a result of the increased amount of imidazole that those rats received, rather than to an effect of indomethacin. Therefore, the rats in group V were subjected to a similar protocol to that used in group IV, except that, insted of indomethacin, they were infused with an equivalent volume of only the buffer that was used as the indomethacin vehicle. As shown in Table II, elimination of the indomethacin also eliminated the deterioration in renal function during the second imidazole infusion period.

Finally, inasmuch as imidazole treatment increased CIn (but not CPAH) in the CK as well as the POK in group II rats, it is possible that imidazole is a nonspecific vasodilator, and that its beneficial effect in the POK was not related to any consequence of ureteral obstruction. To test this possibility, the normal healthy rats of group VI (not subjected to previous obstruction of the ureter) were studied before and during imidazole infusion. As shown on the right side of Table II, neither CIn nor CPAH were significantly increased by such treatment.

Because of the known effect of imidazole, in massive doses, to produce marked hypocalcemia (21), plasma calcium was measured in seven rats each in groups II and IV. Plasma calcium in group II rats before imidazole treatment, 10.15±0.14 mg/dl, decreased ($P < 0.05$) to a value of 9.52±0.23 after completing the imidazole infusion. Plasma calcium after the first imidazole infusion in group IV, 9.50±0.28, also decreased ($P < 0.05$) when measured at the end of the second imidazole period (8.56±0.30). Although statistically significant, these values are considerably greater than the values for plasma calcium observed after 4 h of imidazole infusion (5.41±0.43 mg/dl) at which time blood pressure began to decrease and renal and cardiovascular function deteriorated.

The data in Table II suggest that, although selective inhibition of the vasoconstrictor TXA2 improves renal function in the POK, this improvement depends, in some part, on the continued production of some prostaglandin, presumably a vasodilator. These data do not, however, identify the source of the residual vasoconstriction observed in the POK after the production of both types of arachidonic acid metabolites have been blocked by indomethacin treatment. In an attempt to

[3] Unpublished observations.

TABLE III

*Effect of an Intraarterial Infusion of Either Imidazole (Imid; 10 Rats) or Urea (10 Rats) on Renal Hemodynamics and the Excretion of Salt and Water after the Release of Ureteral Obstruction**

	U/P In‡		V		CIn		CPAH		BP	
	Imid	Urea	Imid	Urea	Imid	Urea	Imid	Urea	Imid	Urea
			µl/kg/min		*ml/kg/min*		*ml/kg/min*		*mm Hg*	
POK										
Pre	122	29	7.7	16.1	0.48	0.46	2.21	1.57	125	135
	±23	±5	±3.0	±2.1	±0.09	±0.12	±0.48	±0.40	±6	±5
Post	213	42	23.3	14.5	2.16	0.56	11.45	1.43	140	130
	±45	±8	±8.2	±2.6	±0.31	±0.10	±2.45	±0.23	±6	±4
P	<0.02	<0.05	NS	NS	<0.001	NS	<0.01	NS	<0.05	NS
CK										
Pre	296	185	15.9	36.8	4.20	4.69	10.17	8.95		
	±42	±36	±2.3	±7.8	±0.43	±0.46	±0.92	±1.04		
Post	114	220	64.4	24.1	5.16	5.03	11.59	7.56		
	±12	±25	±16.6	±2.2	±0.32	±0.49	±1.31	±0.98		
P	<0.001	NS	<0.01	NS	<0.05	NS	NS	NS		
	UNaV		CNa/CIn		U/P Osm		COsm/CIn		P Osm	
	µeq/min/kg		*%*				*%*		*mosm/kg H_2O*	
POK										
Pre	0.21	1.98	0.70	5.06	1.36	1.09	1.69	7.65	298	299
	±0.03	±0.28	±0.27	±1.21	±0.08	±0.02	±0.34	±2.18	±3	±1
Post	1.14	1.73	0.75	2.97	1.74	1.11	1.60	3.65	300	301
	±0.20	±0.38	±0.27	±0.69	±0.11	±0.02	±0.51	±0.70	±3	±1
P	<0.001	NS	NS	NS	<0.001	NS	NS	NS	NS	NS
CK										
Pre	2.09	3.70	0.32	0.56	5.37	4.66	2.12	2.88		
	±0.33	±0.97	±0.09	±0.15	±0.40	±0.78	±0.22	±0.25		
Post	5.52	2.20	1.36	0.32	3.56	5.61	3.79	2.63		
	±0.51	±0.57	±0.31	±0.07	±0.25	±0.54	±0.50	±0.14		
P	<0.001	NS	<0.01	NS	<0.001	NS	<0.01	NS		

* Values are means±SE.

‡ *Abbreviations used in this table*: U/P In, ratio of urine to plasma inulin concentration; V, urine flow rate; COsm, clearance rate of osmoles; CNa/CIn, fraction of filtered sodium excreted; BP, blood pressure; UNaV, absolute excretion rate for sodium; U/P Osm, ratio of urine to plasma osmolality; P Osm, plasma osmolality; Pre, before treatment; Post, after treatment; NS, $P > 0.05$.

characterize other vasoconstrictors that might act on the POK, we investigated the possible role of the renin-angiotensin system. These data are shown in Table IV and Fig. 4.

Fig. 4 demonstrates the effect of infusing the AII antagonist 1-sarcosine, 8-alanine, AII (Saralasin) into rats either before (rats E and F) or after (rats A, B, C, D, and G) releasing UUO. When infused after the release of UUO, renal function was improved in the POK in only one rat (rat C) by doses of 2 and 5 µg/kg per min. In all other animals, both CIn and CPAH deteriorated when the drug was infused. These changes in function of the POK were not associated with significant changes in either function of the CK or in blood pressure. When Saralasin was infused before the release of UUO and continued during the hour immediately after release, as well as during the first clearance period, neither a 5-µg/kg per min dose (rat E) nor a 2-µg/kg per min dose (rat F) appeared to be beneficial as compared with the values obtained after the Saralasin infusion was stopped.

These data do not establish a clear role for AII as a vasoconstrictor in the POK. However, because of emerging evidence suggesting that Saralasin may have agonistic properties (22), further studies to characterize the role of the renin-angiotensin system in the POK were conducted, using the AI-converting enzyme inhibitor, SQ 14225 (Captopril). These data are presented in Table IV.

In group VIII rats, Captopril was infused shortly before UUO was produced. Data from these animals are compared with values observed in rats, matched for

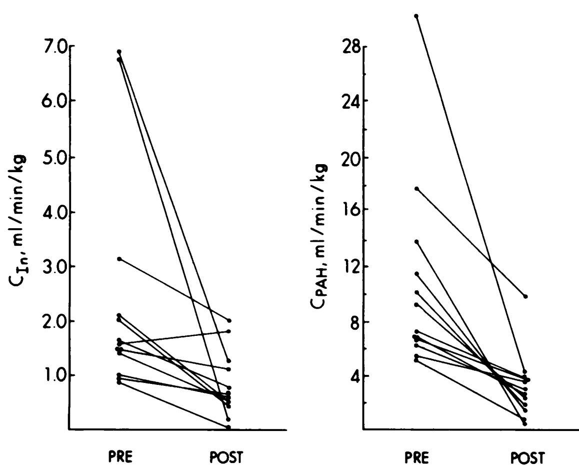

FIGURE 3 CIn and CPAH in the POK during inhibition of thromboxane synthetase with imidazole (Pre), and subsequently after (Post) inhibition of both cyclooxygenase with indomethacin and thromboxane synthetase with imidazole.

weight with group VIII rats, that were infused with an equal volume of the vehicle used to dissolve the Captopril (group IX). Both CIn and CPAH were significantly better in the rats treated with Captopril than in those rats treated with the vehicle only. Pretreatment with Captopril did not affect renal function in the CK.

To use animals as their own control when studying the effects of Captopril, an additional group of rats (group X) was studied in the absence of any drug treatment, and again after Captopril treatment. As in the results in groups VIII and IX, Captopril treatment was accompanied by significant improvement in both CIn and CPAH in the POK, with values in the CK being unchanged.

These data suggest that AII might, indeed, be an important vasoconstrictor in the POK. However, the AI-converting enzyme (kininase II) is relatively nonspecific with many actions, including the degradation of bradykinin and other vasodilator kinins (14). Therefore, the improvement in renal function which followed Captopril treatment might have resulted from decreased degradation of kinins rather than decreased production of AII. To clarify this possibility, two further groups of animals were studied.

Group XI experiments resembled those of group X in that renal function was studied after release of UUO in the absence of drug treatment (first period), then again after Captopril administration (second period). Observations were continued during a third period, in which the rats were additionally treated with carboxypeptidase B, an enzyme that destroys the vasodilator kinins (15), but does not affect AI or AII.

As shown in Table IV, the values for both CIn and CPAH were significantly increased by Captopril treatment in the rats in group XI as compared with the values observed in these animals during the prior period with no treatment. Table IV further shows that the addition of carboxypeptidase B treatment did not diminish these beneficial effects of Captopril in the POK, but rather, appeared to augment them. CIn and CPAH in the POK were both slightly, though not significantly, greater during combined treatment with both agents, than during Captopril alone. In the CK, Captopril treatment increased CIn ($P < 0.02$), and this increase was further augmented ($P < 0.02$) by the addition of carboxypeptidase B treatment. In both the POK and CK, the values for both CIn and CPAH during the final period (carboxypeptidase B) were significantly greater than the values observed during the initial period in which no drugs were administered.

Because the administration of carboxypeptidase B may have other effects in addition to the inactivation of kinins, an alternate method of reducing bradykinin levels was performed in the rats in group XII. In these animals, again after an initial period without pharmacological manipulation, and a second period of Captopril treatment, the rats were treated during a third period with the kallikrein inhibitor, Trasylol, to block the production of the kinins (16, 17).

Similar to the results in groups X and XI, Captopril treatment again significantly increased both CIn and CPAH in the POK in group XII. After the addition of Trasylol, CIn improved even further ($P < 0.02$) in the POK. No values for CPAH are presented during this third period because the values during the first 30 min increased sharply and then fell dramatically during the second 30 min to levels which were similar to CIn in both kidneys. Whether this fall in CPAH represents a true decrease in renal blood flow, an effect on PAH secretion, or an artifact resulting from the effect of rapidly changing blood concentrations is not clear. Therefore, the effect on renal blood flow of Captopril and Trasylol in combination remains problematic.

DISCUSSION

After the release of as little as 24 h of UUO, there is intense renal vasoconstriction in the POK, accompanied by a decrease in renal blood flow and glomerular filtration rate. In a previous study using a vascular injection technique, we demonstrated (2) that this postobstructive vasoconstriction tended to spare the medulla and to involve the cortex heterogeneously, in that there were relatively large areas of the cortex in which perfusion beyond interlobular arteries could not be demonstrated. Because anatomic hydronephrosis is relatively slight at this time, and because both acute extracellular volume expansion (3) and infusion of vasodilators (4) have been shown to improve renal blood flow in the POK, these findings suggest that the

Table IV

Effect of Blocking the Production of A II with the Converting Enzyme Inhibitor, Captopril, on Renal Function after the Release of 24 h of UUO, and the Inability of a Bradykinin-Inactivating Agent (Carboxypeptidase B) or a Kallikrein-Inhibiting Agent (Trasylol) to Block the Effect

Group	POK						CK					
	Hours after release of the ureter						Hours after release of the ureter					
h......	1		2		3	4	1		2		3	4
VIII (Pretreated with Captopril)												
n = 6	<----No treatment------>						<---No treatment----->					
	CIn	CPAH					CIn	CPAH				
	1.87‡	6.75					5.85	13.16				
	±0.34	±1.16					±0.70	±1.44				
IX (Pretreated with Captopril vehicle)												
n = 6	<----No treatment------>						<---No treatment----->					
	CIn	CPAH					CIn	CPAH				
	0.59	2.30					5.64	13.91				
	±0.13	±0.88					±0.31	±1.61				
P′	<0.001	<0.02					NS	NS				
X n = 20	<----No treatment------>		<--------Captopril-------->				<---No treatment----->		<--------Captopril-------->			
	CIn	CPAH	CIn	CPAH			CIn	CPAH	CIn	CPAH		
	0.95	4.16	1.93	7.29			4.87	11.69	4.89	12.88		
	±0.19	±0.55	±0.30	±0.93			±0.26	±1.07	±0.39	±1.01		
P			<0.01	<0.01					NS	NS		
XI n = 8	<----No treatment------>		<--------Captopril-------->		<------Carboxy-peptidase B---->		<---No treatment----->		<--------Captopril-------->		<------Carboxy-peptidase B---->	
	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH	CIn	CPAH
	0.49	2.85	1.14	4.72	1.54	5.51	4.46	12.59	5.69	13.71	7.23	17.69
	±0.12	±0.72	±0.18	±0.70	±0.28	±1.18	±0.32	±1.21	±0.44	±1.06	±0.64	±2.30
P			<0.01	<0.02	NS	NS			<0.02	NS	<0.02	NS
P″					<0.01	<0.05					<0.01	<0.05
XII n = 11	<----No treatment------>		<--------Captopril-------->		<--------Trasylol------>		<---No treatment----->		<--------Captopril-------->		<--------Trasylol------>	
	CIn	CPAH	CIn	CPAH	CIn	CPAH§	CIn	CPAH	CIn	CPAH	CIn	CPAH
	0.56	3.33	1.22	5.88	2.11	—	4.95	12.94	5.16	12.14	5.91	—
	±0.14	±0.77	±0.22	±1.40	±0.42	—	±0.18	±0.51	±0.15	±0.63	±0.56	—
P			<0.01	<0.05	<0.02	—			NS	NS	NS	—
P″					<0.01	—					NS	—

* P, probability of significant difference compared with previous treatment period; P', difference of group IX compared with group VIII; P'', difference of values in hours 3–4 compared with values in hours 1–2; NS, $P > 0.05$.

‡ Values are ml/kg per min, means±SE.

§ No P-aminohippuric acid data are reported because of potential methodological errors, see text.

vasoconstriction observed after the release of 24 h of UUO is predominantly functional rather than structural. The present studies were undertaken in an attempt to identify possible roles for prostaglandins, thromboxanes, and the renin-angiotensin system in these alterations of renal hemodynamics.

Other investigators (7–9) have provided evidence that prostaglandins and thromboxanes might be impor-

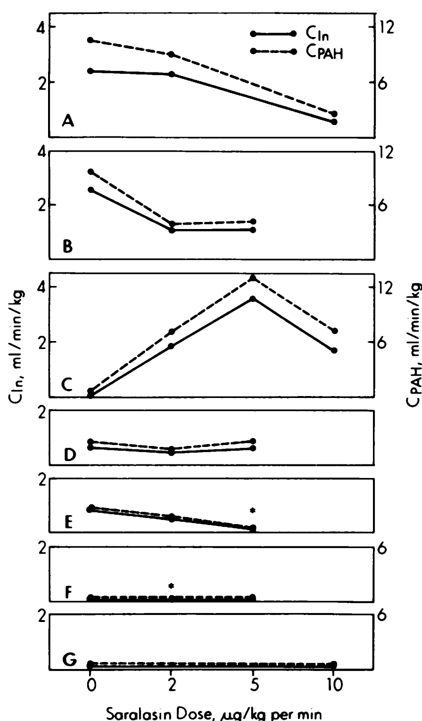

FIGURE 4 The effects of infusing various doses of the AII antagonist Saralasin on the CIn and CPAH by the POK in seven rats. In rats E and F (*), Saralasin was infused both before and after release of obstruction; in the other five rats, Saralasin was infused only after release.

tant mediators of postobstructive renal function. Nishikawa et al. (7) reported that, after 72 h of UUO, the in vitro perfused hydronephrotic rabbit kidney demonstrates increased production of PGE2 when stimulated with either bradykinin or AII, as compared with the intact CK. Subsequently, this same group demonstrated that the in vitro perfused hydronephrotic rabbit kidney also exhibits increased production of TXA2, in response to infusion of bradykinin (8, 9).

In previous attempts to define a role for prostaglandins after UUO in rats, both Edwards et al. (23) and we (24) treated animals with indomethacin at the time UUO was produced and, again, just before it was released. Such treatment has been shown to significantly decrease rat renal prostaglandin synthesis (23); however, in both studies, it failed to improve function in the POK. One explanation for this lack of improvement is that indomethacin, in the dosages used, may have impaired cardiac function during the 24 h of UUO. However, administration of indomethacin only as an acute injection of the drug after the release of UUO in the present studies (group I) also failed to improve renal function.

Although these results can be interpreted in several ways, one interpretation is that indomethacin inhibited the synthesis of both a vasodilator and a vasoconstrictor, and thus, had no net beneficial effect on the POK. To examine this possibility, we infused imidazole, an inhibitor of thromboxane synthetase (8, 9), into rats after the release of UUO (group II). Our finding that imidazole infusion resulted in a significant improvement in renal function in the POK is compatible with a role for TXA2 as a renal vasoconstrictor in the POK.

Although the reversal of vasoconstriction in the POK by imidazole may have resulted from blockade of thromboxane synthesis, other explanations are possible. First, imidazole infusion was accompanied by an increase in CIn in the intact CK, raising the possibility that imidazole might be a nonspecific renal vasodilator. This appears unlikely, however, because imidazole had no measurable effect on renal hemodynamics in normal rats (group VI).

Second, because acute saline expansion has been shown to decrease vasoconstriction in the POK (3), it is possible that part of the beneficial effect of imidazole treatment was a nonspecific consequence of the osmotic or volume load of the infusion. We examined this possibility by infusing urea into rats (group III) in a solution of the same volume, osmolality, and pH as that used for imidazole. Urea was chosen because it is of similar molecular weight and, like imidazole, is not confined to the extracellular space. Our finding that urea infusion did not improve function of the POK (Table III) is evidence against an important nonspecific osmotic or volume effect of the imidazole infusion. Although we previously reported that urea loading improved POK function in rats, the concentration of urea in the infusion solution in those experiments was 10 times greater than in the present studies (25). These results, then, support the concept that imidazole exerted its effect of reversing vasoconstriction in the POK primarily by inhibiting the renal synthesis of vasoconstrictor TXA2.

This beneficial effect of imidazole in the POK was abolished by the addition of indomethacin treatment (group IV) while the imidazole infusion was extended. This finding suggests that the ability of imidazole to improve POK function depends on prostaglandin metabolism being intact except for the blockade of thromboxane synthesis. However, there are alternative interpretations of these results. First, imidazole is a stimulator of phosphodiesterase (26), whereas indomethacin may be a phosphodiesterase inhibitor (27). Thus, it is possible that the beneficial effects of imidazole, and their reversal by indomethacin, might be mediated by alterations in cyclic nucleotide metabolism. Plasma concentrations of imidazole, measured in the present study, did not exceed 2.5 mM. By comparison, imidazole levels as high as 6 mM have been shown to inhibit

platelet thromboxane synthesis without altering either basal or stimulated cyclic AMP levels (11). Although these data do not exclude the possibility that altered cyclic nucleotide metabolism might contribute to the improvement in POK function induced by imidazole, they suggest that other mechanisms are more important.

Second, imidazole in large doses may lead to hypocalcemia (21), an effect possibly related to stimulation of phosphodiesterase. Indeed, we have observed[3] that imidazole, when infused into normal rats continuously for 4 h or more, often leads to marked hypocalcemia and tetany. Plasma imidazole concentrations in these rats generally exceeded 8 mM, compared with levels of <2.5 mM in group II and group IV rats in the present study. This finding, in itself, does not remove the possibility that the modest decrease in plasma calcium noted in group IV rats could have contributed to the depression of POK function after indomethacin administration. Such a possibility seems unlikely, however, because renal function did not decrease in either the POK or CK of group V rats given the same total dose of imidazole, but without indomethacin. Although CPAH did decrease in the CK of group IV rats during the second imidazole period, CPAH also decreased in group I rats after indomethacin treatment alone. Both of these decreases seem likely to be an effect of indomethacin per se, not of hypocalcemia. This interpretation is supported by the observations of Terragno et al. (28) that indomethacin treatment decreases renal blood flow in anesthetized, surgically stressed animals.

Considered together, the data from groups I to VI suggest that both vasodilator and vasoconstrictor metabolites of arachidonic acid are important in postobstructive renal hemodynamics in the rat; they do not, of course, identify the compounds responsible. Although the beneficial response to imidazole suggests that TXA2 is an important determinant of function in the POK, the nature of the prostaglandin vasodilator is less clear. The hydronephrotic rabbit kidney produces increased amounts of PGE2—a compound which is a vasodilator in that species. However, extrapolation of these data to infer that PGE2 is a vasodilator in the POK of the rat, is probably not warranted at the present time for at least two reasons. First, increased PGE2 production by the hydronephrotic rat kidney has not yet been demonstrated. Second, the preponderance of data at the present time suggests that PGE2 is actually a vasoconstrictor in the rat (20, 29, 30). Thus, the present data suggest that, although its identity is not yet established, the interplay between some prostaglandin vasodilator and TXA2 is important in determining function after release of 24 h of UUO in the rat.

The observation that renal function deteriorated when imidazole infusion was resumed after indomethacin treatment also implies that there is some nonprostaglandin vasoconstrictor acting on the POK. Because it is known that acute ureteral obstruction is accompanied by an increase in renin production (5), we investigated the possibility that AII was a vasoconstrictor in the POK.

We first infused the AII antagonist, Saralasin. As shown in Fig. 2, except for one rat out of seven, there was no evidence that Saralasin improved renal hemodynamics in the POK. However, because Saralasin may itself act as a renal vasoconstrictor (22) as a result of its angiotensin agonistic properties, we further evaluated the role of AII by infusing the converting enzyme inhibitor, Captopril.

When postobstructive renal function in animals pretreated with Captopril before the production of UUO (group VIII) was compared to function of UUO rats similarly pretreated with only the Captopril vehicle (group IX), renal function was significantly improved in the former group. In addition to the group VIII and group IX studies, in which AII production was inhibited by Captopril during the 24 h of UUO, we also examined the effects of Captopril given after UUO was released (group X rats), and again observed that Captopril substantially improved renal function in the POK. Thus, the data from groups VIII–X indicate that Captopril improves renal function in the POK (but not CK) if given either before UUO is produced, or after it is released.

Converting enzyme, which is blocked by Captopril, is a relatively nonspecific dipeptidylpeptidase. In addition to converting AI to AII, it also degrades bradykinin and other vasoactive kinins, such as kallidin (14). Thus, the beneficial effect of Captopril treatment might have resulted from an increase in the level of the vasodilator kinins, rather than a decreased production of the vasoconstrictor AII.

To examine the possible role of increased kinins in the improvement in function of the POK, two different procedures were used to decrease kinin levels. First, the enzyme carboxypeptidase B, which cleaves a basic carboxyl terminal amino acid, was infused intravenously. All the vasoactive kinins have a carboxyl terminal arginine, cleavage of which will inactivate the peptide (15). Thus, this enzyme should lower kinin levels, even if they had been previously increased by the inhibition of kininase II (converting enzyme) with Captopril. Indeed, Erdos and colleagues have shown that the infusion of carboxypeptidase B blocks the effect of intravenously infused bradykinin when tested in the rat, cat (31), or guinea pig (15).

A second method used to examine the possible contribution of an increase in kinin levels to the effect of Captopril on the POK was to infuse Trasylol. This peptide, isolated from bovine lung, has been shown to block the effect of both trypsin and kallikrein (17), enzymes that are both capable of generating kinins.

The observation that renal function does not deterio-

rate with either carboxypeptidase B or Trasylol, but actually improves, suggests strongly that increased kinin activity was not the explanation for the observed increase in renal function that follows Captopril treatment. These data do not, of course, explain why renal function should improve further when agents such as carboxypeptidase B or Trasylol, which are capable of decreasing kinin levels are infused. Irrespective of the unanticipated beneficial effect of these two agents, the data do demonstrate that the reversal of vasoconstriction in the POK after Captopril administration does not depend on an increase in the activity of vasodilator kinins. It would appear, rather, that the major effect of Captopril was to inhibit AII production. Thus, it seems reasonable to conclude that an increased level of intrarenal AII activity explains the nonprostaglandin component of the vasoconstriction observed in the POK.

Taken together, the data presented herein suggest that both metabolites of arachidonic acid and the renin-angiotensin system make important contributions to the determination of postobstructive renal function after the release of 24 h of UUO in the rat. That these two intrarenal hormones should interact in determining renal function is not surprising in that some prostaglandins have been shown to stimulate renin production (32), whereas AII is a known activtor of the phospholipase, which, by releasing arachidonic acid from membrane bound phospholipids, controls the rate-limiting step in prostaglandin and thromboxane biosynthesis. The data also suggest that both vasoconstrictor (TXA2) and vasodilator metabolites of arachidonic acid are of importance, although the identity of the latter has not been established for the rat. Finally, given that pharmacological agents are capable of modulating the effect of these hormones, the present findings suggest areas of exploration for therapeutic manipulation of postobstructive renal function in man.

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