Influence of Prostaglandins on Papillary Blood Flow and Pressure-Natriuretic Response

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The present study examined whether renal prostaglandins influence the pressure-natriuretic response by altering medullary hemodynamics or renal interstitial pressure. The diuretic and natriuretic responses to changes in renal perfusion pressure were compared in control rats (n=15) and in rats receiving either meclofenamate (2 mg/kg, n=9) or indomethacin (2 mg/kg, n=4). In control rats, urine flow and sodium excretion increased from 10 ±2 to 118 ±10 µl/min/g kidney wt and from 1.8±0.3 to 21.0±1.5 µeq/min/g kidney wt, respectively, when renal perfusion pressure was increased from 109 to 167 mm Hg. Urinary excretion of prostaglandin E₂ and thromboxane B₂ increased significantly by 152% and 190%, respectively. Meclofenamate lowered thromboxane B₂ and prostaglandin E₂ excretion and prevented the increase in eicosanoid excretion produced by elevations in perfusion pressure. The pressure-diuretic and pressure-natriuretic responses of rats given meclofenamate or indomethacin were approximately half of those observed in the control rats. Papillary blood flow increased 21% and renal interstitial pressure rose from 5.0±0.7 to 8.2±0.7 mm Hg in control rats when pressure was elevated from 100 to 150 mm Hg. Meclofenamate and indomethacin lowered papillary blood flow and renal interstitial pressure and blunted the increases in these values produced by elevations in perfusion pressure. These results support the view that renal prostaglandins modulate the pressure-natriuresis relation by altering renal medullary hemodynamics and suggest that an intact renal prostaglandin system is necessary for the full expression of the medullary hemodynamic and natriuretic responses to increases in renal perfusion pressure. (Hypertension 1990;15:29-35)

Carmines et al¹ and Haas et al² have reported that cyclooxygenase inhibitors attenuate the natriuretic response to elevations in renal perfusion pressure (RPP); however, the mechanism by which prostaglandins influence this response is unknown. Romero and Knox³ proposed that changes in renal prostaglandin levels may mediate the pressure-natriuretic response because elevations in RPP¹ and renal interstitial hydrostatic pressure (RIHP)⁴ increase the urinary excretion of prostaglandin E₂ (PGE₂), and PGE₂ inhibits tubular reabsorption of sodium.⁵-⁷ On the other hand, the pressure-natriuretic response is associated with inhibition of sodium reabsorption in the proximal tubule or thin descending loop of Henle of deep nephrons,⁸,⁹ and it is difficult to explain how elevations in RPP alter PGE₂ levels in the juxtamedullary cortex of the kidney. Moreover, renal prostaglandins are primarily thought to inhibit tubular reabsorption of sodium in the thick ascending loop of Henle and the collecting duct.¹⁰-¹²

Other studies have indicated that the pressure-natriuretic response is associated with changes in renal medullary hemodynamics and RIHP.¹³ In view of evidence indicating that prostaglandins regulate the intrarenal distribution of cortical and papillary blood flow,¹⁴-¹⁶ the present study examined whether cyclooxygenase inhibitors blunt the pressure-natriuretic response by altering renal medullary vascular resistance and the changes in RIHP produced by elevations in RPP.

Methods

Experiments were performed on 78 male, Sprague-Dawley rats (250–350 g) purchased from Harlan Industries (Madison, Wisconsin). Food and water were allowed ad libitum before the study.
Surgical Preparation for Clearance Experiments

The rats were prepared for study of the pressure-natriuretic response as described previously. They were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg) and maintained at 37°C. The carotid and femoral arteries were cannulated for measurement of RPP. Cannulas were placed in the jugular vein for infusions and in the ureter for collection of urine. A 2 mm flow probe was placed around the left renal artery for measurement of renal blood flow (RBF) with an electromagnetic flowmeter (model 501, Carolina Instruments, King, North Carolina). Adjustable clamps were placed around the abdominal aorta above and below the kidney, and ligatures were placed around the coeliac and mesenteric arteries so that RPP could be varied by adjustment of peripheral vascular resistance.

To prevent baroreceptor reflex changes in renal nerve activity from influencing the renal response to changes in perfusion pressure, the left kidney was acutely denervated by stripping the renal artery and coating the area with a 10% solution of phenol in ethanol. We have reported that this procedure reduced the norepinephrine concentration of renal tissue by more than 97%. Because changes in arterial pressure also alter plasma levels of angiotensin II, aldosterone, vasopressin, and catecholamines, the circulating levels of these hormones were fixed by intravenous infusion of aldosterone (66 ng/kg/min), cortisol (33 ng/kg/min), vasopressin (0.17 ng/kg/min), and norepinephrine (333 ng/kg/min). The drugs were dissolved in a 0.9% sodium chloride solution containing 1% bovine serum albumin. Infusion of the hormone cocktail was initiated during surgery and was continued at a rate of 33 μl/min/100 g body wt throughout the experiment. [3H]Inulin (1 μCi/ml) was included in the infusion solution for measurement of glomerular filtration rate (GFR). In previous studies, we demonstrated that infusion of this hormone cocktail in rats raised plasma concentration of aldosterone to more than 200 ng/dl and elevated plasma vasopressin and norepinephrine concentrations to nonpressor levels, about five times the values found in conscious animals. Plasma renin activity and plasma levels of angiotensin II and atrial natriuretic factor in the rats infused with hormone cocktail were similar to values reported in conscious rats.

Protocol 1: Effect of Cyclooxygenase Inhibitors on Pressure-Natriuretic Response

Experiments were performed on 15 control rats given vehicle, and in rats given a 2 mg/kg intravenous dose of either meclofenamate (n=9) or indomethacin (n=4). We have previously reported that the administration of these doses of indomethacin and meclofenamate to rats in vivo reduced the urinary excretion of PGE2 and completely blocked the ability of renal medullary tissue to synthesize PGE2 in vitro. Thirty minutes after the rats received vehicle or the cyclooxygenase inhibitors, RPP was lowered to 100 mm Hg by aortic occlusion; then, after a 10-minute equilibration period, urine flow, sodium excretion, GFR, and RBF were measured during two 30-minute periods. Then, RPP was returned to control levels (approximately 125 mm Hg), and after a 10-minute equilibration period, urine and plasma samples were again collected during two 15-minute periods. RPP was then increased to 150 mm Hg by occlusion of the mesenteric and coeliac arteries and the aorta below the kidneys, and after a 10-minute equilibration period, samples were again collected during two 15-minute periods.

Urine flow was determined gravimetrically. [3H]Inulin concentrations of the samples were determined with a liquid scintillation spectrophotometer (model 2450, Packard Instr. Co., Downers Grove, Illinois). Sodium and potassium concentrations were determined with a flame photometer (model 143, Instrumentation Laboratories, Lexington, Massachusetts). GFR was calculated as the urine-to-plasma inulin concentration ratio times urine flow. GFR, RBF, urine flow, and electrolyte excretions were factored per gram kidney weight.

Protocol 2: Urinary Excretion of Prostaglandin E2 and Thromboxane B2

The effects of changes in RPP on the urinary excretion of (PGE2) and thromboxane B2 (TXB2) were studied in six control rats and five rats given meclofenamate. The rats were surgically prepared for clearance experiments except that [3H]inulin was not infused because it would interfere with the radioimmunoassays. Thirty minutes after the rats received meclofenamate or vehicle, RPP was lowered to 100 mm Hg, and a urine sample was collected for measurement of PGE2 and TXB2 excretion during a 30-minute control period. RPP was then increased to 125 and 150 mm Hg, and urine samples were sequentially collected during two 30-minute experimental periods.

Urinary concentrations of PGE2 and TXB2 were measured after their extraction and separation by radioimmunoassay with reverse-phase C18 preparatory columns (Sep-Pak, Millipore Corp., Bedford, Massachusetts) as described previously. The PGE2 antisera was obtained from the Pasteur Institute (Paris, France) and the TXB2 antisera was a gift from Dr. William Campbell (University of Texas Health Science Center, Dallas, Texas). In previous studies, this antibody has been reported to exhibit a cross-reactivity of less than 0.01% with other arachidonic acid metabolites.

Protocol 3: Effect of Cyclooxygenase Inhibitors on Renal Interstitial Hydrostatic Pressure

These experiments were performed on seven control rats, six rats given meclofenamate, and five rats that received indomethacin. RIHP was measured with the implanted capsule technique as described previously. A small hole, 1 mm in diameter and 3 mm deep, was created in the renal cortex with an
electrocautery needle. After the bleeding was stopped, the interstitial capsule was inserted into the hole. Cyanoacrylate adhesive was placed on the surface of the kidney to prevent leaks. The catheter was flushed with 20 μl saline, and the pressure in the capsule was continuously recorded with a transducer (model P23, Gould Statham Instruments, Cleveland, Ohio) calibrated between 0 and 20 mm Hg.

After surgery and a 1-hour equilibration period, RIHP, RBF, and RPP were measured during a 15-minute control period. The rats were then given vehicle or the cyclooxygenase inhibitors, and 30 minutes later, these values were measured during a 15-minute experimental period. After this period, the relation between RIHP and RPP was determined. RPP was elevated to 150 mm Hg by tying off the mesenteric and coeliac arteries and then sequentially reduced to 50 mm Hg in increments of 10 mm Hg. The kidney was perfused for 3 minutes at each level of pressure and the steady-state values for RBF and RIHP were recorded.

Protocol 4: Effect of Cyclooxygenase Inhibitors on Cortical and Papillary Blood Flow

The relation between cortical and papillary blood flow and RPP were characterized before and after the rats were given vehicle or cyclooxygenase inhibitors. One week before an experiment, these animals were anesthetized with ketamine (100 mg/kg) and acepromazine (1 mg/kg). A small amount of renal cortical tissue on the dorsal surface of the left kidney was removed to allow for exposure of the papilla. On the day of the acute experiment, the left kidney was immobilized in a holder and the papilla was exposed by excision of the ureter.

Cortical and papillary blood flows were measured using a laser-Doppler flowmeter (model Pfdl, Perimed KB, Stockholm, Sweden) as described previously. RPP was first increased (approximately 25 mm Hg) by occlusion of the coeliac and mesenteric arteries, and cortical and papillary blood flows were measured as RPP was lowered from 150 to 50 mm Hg in increments of 15 mm Hg. RPP was then returned to 150 mm Hg, and the rats were given 2 mg/kg meclofenamate (n=7), 2 mg/kg indomethacin (n=4), 5 mg/kg naproxen (n=4), or vehicle (n=4). After a 30-minute equilibration period, the relation between cortical and papillary blood flows and RPP were redetermined.

Statistical Methods

Data are presented as mean±1 SEM. The significance of differences in values measured at different levels of RPP was evaluated with an analysis of variance for repeated measures. The significance of differences in measured values between groups of rats was evaluated with a two-way analysis of variance and a Duncan multiple range test. The curves relating blood flow and RPP were determined with a third-order fitting procedure. A probability level of p<0.05 (two-tailed test) was considered significant.

Results

The relation between urine flow, sodium excretion, and RPP were similar in the rats given indomethacin or meclofenamate. In meclofenamate-treated rats, urine flow and sodium excretion increased from 9.7±2.6 to 58.5±5.6 μl/min/g kidney wt and from 1.8±0.5 to 11.5±1.1 μeq/min/g kidney wt, respectively, in response to an elevation in RPP from 104 to 159 mm Hg. In indomethacin-treated rats, urine flow and sodium excretion increased from 4.9±1.6 to 64.9±14.5 μl/min/g kidney wt and from 1.4±0.5 to 11.8±2.8 μeq/min/g kidney wt in response to a similar elevation in RPP. There were no significant differences in the pressure-diuretic and pressure-natriuretic responses in the indomethacin- and meclofenamate-treated rats; therefore, the data from these two groups were pooled and compared with the results obtained in the control rats given vehicle alone (Figures 1 and 2).

In control rats, urine flow and sodium excretion increased 10-fold in response to an elevation in RPP from 110 to 160 mm Hg (Figure 1). In rats pretreated with either indomethacin or meclofenamate, the increases in urine flow and sodium excretion were about half of those seen in the control rats. Renal blood and GFR (Figure 2) were autoregulated in both the control rats and rats given the cyclooxygen-
The effects of changes in RPP on the urinary excretion of PGE\(_2\) and TXB\(_2\) in the control and meclofenamate-treated rats are presented in Figure 3. In the control rats, urinary excretion of TXB\(_2\) and PGE\(_2\) increased significantly by 190% and 152%, respectively, after RPP was elevated from 100 to 150 mm Hg. Urinary excretion of TXB\(_2\) and PGE\(_2\) was significantly lower in the meclofenamate-treated rats by about 60%. Moreover, the increases in TXB\(_2\) and PGE\(_2\) excretion produced by elevations in RPP were prevented by meclofenamate.

The effect of meclofenamate on the relation between RIHP and RPP is presented in Figure 4. In the control rats, RIHP rose from 5.0±0.6 to 8.2±0.7 mm Hg as RPP was increased from 100 to 150 mm Hg. In the rats given either indomethacin or meclofenamate, RIHP fell significantly by about 3 mm Hg over a 15-minute period, while RBF and arterial pressure were unaltered. As a consequence, the relation between RIHP and RPP was shifted toward higher pressures. As can be seen in Figure 4, RIHP was significantly lower in meclofenamate-treated rats than in control animals throughout the range of RPP from 100 to 150 mm Hg. Moreover, RIHP increased by only 1.6±0.3 mm Hg when RPP was elevated from 100 to 150 mm Hg in meclofenamate-treated rats. This increase in RIHP was significantly less than the rise of 3.2±0.5 mm Hg observed in the vehicle-treated rats.

The effects of indomethacin and meclofenamate on the cortical and papillary blood flow are presented in Figures 5 and 6. Neither drug altered outer cortical blood flow. The relation between cortical blood flow and RPP were similar before and after indomethacin (Figure 5), meclofenamate (Figure 6), or naproxen (data not shown). In contrast, all three cyclooxygenase inhibitors reduced papillary blood flow. Papillary blood flow fell to 65±12% of control levels within 15...

\[\text{FIGURE 2. Line graphs showing effect of cyclooxygenase inhibitors on renal blood flow and glomerular filtration rate.} \]
\[\text{* Indicates significant difference from value (•) measured at control level of arterial pressure.} \]
\[\text{† Indicates significant difference between values in rats given the cyclooxygenase inhibitors or vehicle. PG, prostaglandin; kwt, kidney weight.} \]

\[\text{FIGURE 3. Line graphs showing urinary excretion of thromboxane B\(_2\) (TXB\(_2\)) and prostaglandin E\(_2\) (PGE\(_2\)) in meclofenamate- and vehicle-treated rats.} \]
\[\text{* Indicates significant difference from value (•) measured at a pressure of 100 mm Hg.} \]
\[\text{† Indicates significant difference between values in meclofenamate- and vehicle-treated rats.} \]

\[\text{FIGURE 4. Line graph showing effect of meclofenamate on relation between renal interstitial hydrostatic pressure and renal perfusion pressure.} \]
\[\text{* Indicates significant difference from value (•) measured at control level of arterial pressure.} \]
\[\text{† Indicates significant difference between values in meclofenamate- and vehicle-treated rats.} \]
minutes after administration of indomethacin, to 56±2% of control levels after meclofenamate, and to 48±3% of control levels after naproxen. Thus, the relation between papillary blood flow and RPP was shifted toward higher pressures by each of the cyclooxygenase inhibitors. As can be seen in Figures 5 and 6, papillary blood flows measured at any RPP after indomethacin or meclofenamate were significantly lower than the flows measured during the control period. In time-control rats, the relation between cortical and papillary blood flow and RPP determined before and after administration of vehicle were not significantly different (data not shown).

Discussion

The influence of eicosanoids on the pressure-natriuretic relation and their role in the long-term control of arterial pressure has not been examined in detail. The recent finding that cyclooxygenase inhibitors attenuate pressure-natriuretic response1-2 indicates that renal prostaglandins influence the relation between sodium excretion and RPP, but their mechanism of action is unknown. Haas et al2 and Romero and Knox3 recently proposed that changes in renal prostaglandin levels may mediate pressure-natriuresis by inhibiting tubular reabsorption in the proximal tubule of deep nephrons. This hypothesis was based on the findings that elevations in RPP or RIHP increase the urinary excretion of PGE2 and that cyclooxygenase inhibitors block the natriuretic response to either maneuver.1,4 The present study confirms that the pressure-natriuretic response is accompanied by a significant increase in the urinary excretion of PGE2, and extends this observation to TXB2 excretion. These changes in PGE2 and TXB2 excretion, however, do not establish that these substances mediate the response. In particular, it is difficult to explain how an increase in intrarenal levels of the vasoconstrictor TXB2 participates in the pressure-natriuretic response. We suspect that the simultaneous increases in TXB2 and PGE2 excretion are a consequence and not the cause of the diuresis and natriuresis. In support of this view is the previous data indicating that eicosanoid excretion may be dependent on urine flow rate.27,28 Moreover, the present finding that meclofenamate blocked the changes in PGE2 and TXB2 excretion but did not prevent the natriuresis produced by elevations in RPP argue against the role of prostaglandins in mediating this response. This conclusion is also supported by the recent finding that inhibitors of prostaglandin synthesis had little effect on the pressure-natriuretic response of dogs pretreated with captopril.29

In view of these findings, the present study examined whether renal prostaglandins might serve as modulators (rather than mediators) of the pressure-natriuretic response by altering renal medullary hemodynamics or RIHP. Our hypothesis was based on previous studies indicating that pressure-natriuresis is associated with changes in renal medullary hemodynamics13 and RIHP.3,24 It was suggested that
elevations in RHP may inhibit tubular reabsorption of water and electrolytes in the proximal tubule of superficial and deep nephrons or in the thin descending loop of Henle of deep nephrons.\textsuperscript{8,9} by altering the passive backleak of ions.\textsuperscript{26} The present results support this interpretation because pressure-natriuresis occurred in the absence of measurable changes in RBF, GFR, or cortical blood flow and was associated with elevations in papillary blood flow and RHP. On the other hand, the present data does not exclude the possibility that the blunting of the pressure-natriuretic response and the changes in renal medullary hemodynamics and RHP after administration of cyclooxygenase inhibitors were unrelated. For example, an increase in tubular reabsorption of sodium after removal of the inhibitory actions of PGE\textsubscript{2} could explain the shift to the right in the pressure-natriuretic relation after cyclooxygenase inhibitors independent of changes in renal medullary hemodynamics.

Administration of cyclooxygenase inhibitors reduced RHP and blunted the rise in RHP produced by elevations in RPP. The fall in RHP was probably due to the changes in medullary hemodynamics because meclofenamate or indomethacin did not significantly alter RBF, GFR, and cortical blood flow. Our findings are consistent with previous results indicating that changes in RPP are partially transmitted to the vasa recta circulation, and that elevations in RPP produce similar increments in vasa recta capillary pressure and RHP.\textsuperscript{13}

Several studies have indicated that the magnitude of the pressure-natriuretic response is dependent on the basal level of RHP and is correlated with the increment in RHP.\textsuperscript{13,24,30} In the present study, meclofenamate blunted the increase in RHP produced by elevations in RPP and attenuated the pressure-natriuretic response. These effects are similar to those recently reported when the renal capsule of rats was removed to blunt the increase in RHP produced by elevations in RPP\textsuperscript{24} and further support a role for RHP in the control of sodium excretion.

The fall in papillary blood flow after meclofenamate in face of constant RBF and arterial pressure indicates that renal medullary vascular resistance was elevated by inhibition of prostaglandin synthesis. Our findings confirm previous studies indicating that cyclooxygenase inhibitors may selectively reduce blood flow to juxtamedullary nephrons\textsuperscript{14} and the vasa recta circulation\textsuperscript{15,16} and further support a role for prostaglandins in the regulation of medullary blood flow. The link between changes in papillary blood flow, RHP, the pressure-natriuretic response, and the long-term control of arterial pressure remains to be established. The present study suggests that a chronic reduction in renal prostaglandin production might promote the development of hypertension by shifting the pressure-natriuresis relation to the right. In this regard, it is interesting that the development of hypertension in the SHR is accompanied by changes in renal arachidonic acid metabolism,\textsuperscript{22-34} a fall in papillary blood flow,\textsuperscript{2,33,35} and RHP,\textsuperscript{36} and an abnormal pressure-natriuretic response.\textsuperscript{37}

In summary, the pressure-natriuretic response is associated with increases in papillary blood flow, RHP, and the urinary excretion of PGE\textsubscript{2} and TXB\textsubscript{2}. Inhibition of renal prostaglandin synthesis lowered papillary blood flow and RHP and blunted but did not eliminate the pressure-natriuretic response. These findings suggest that renal prostaglandins may modulate the pressure-natriuretic response by influencing RHP through alterations in medullary vascular resistance. An intact renal prostaglandin system appears to be necessary for the full expression of the renal medullary hemodynamic and natriuretic responses to an increase in renal perfusion pressure.

Acknowledgments

We thank C. Smits, M. Kaldunski, and M. Koreleski for their technical assistance with these experiments, Rhoda Adelsen for secretarial assistance, and the Warner Lambert Company (Ann Arbor, Michigan) for providing meclofenamate.

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KEY WORDS • prostaglandins • renal function • blood flow • renal hypertension • kidney
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doi: 10.1161/01.HYP.15.1.29

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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