Role of Renal Papillae in the Regulation of Sodium Excretion During Acute Elevation of Renal Perfusion Pressure in the Rat

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SUMMARY We studied the role of renal papillae in the mechanism of increased sodium excretion during acute increase in mean arterial pressure (MAP). Sodium excretion increased dramatically in normal rats after acute increase in MAP by epinephrine (E) infusion (0.4 μg/min/100g). Glomerular filtration rate (GFR), renal blood flow (RBF), and papillary plasma flow (PPF) remained unchanged after the E administration. To define the role of the medulla in the mechanism of pressure-induced natriuresis, experiments were performed in a group of rats 8 to 12 days after the development of papillary necrosis induced by bromoethylamine hydrobromide. Urinary sodium and fractional sodium excretions were 2.00 ± 0.34 μEq/min and 2.37 ± 0.53% (n = 7), respectively, in papillary necrosis rats infused with saline. Administration of E to papillary necrosis rats, however, failed to increase both urinary sodium (2.89 ± 0.61 μEq/min) and fractional sodium (E_N, 2.82 ± 0.63%, n = 6) excretions despite a marked increase in MAP (129 vs 150 mm Hg, p < 0.01). The RBF increased slightly after E infusion (4.42 vs 3.24 ml/min/100 g, p < 0.05), but the GFR was not different between the control (0.39 ± 0.05 ml/min/100 g, n = 7) and the E-treated rats (0.43 ± 0.06, n = 6). Failure to increase sodium excretion during acute increase in MAP was not due to the decreased GFR, since control rats with bilateral partial nephrectomy were able to increase sodium excretion from 1.92 ± 0.33 to 7.76 ± 1.63 μEq/min (p < 0.01) after E infusion. These findings, therefore, suggest that renal papillae play a major role in the mechanism of natriuresis during acute increase in MAP. (Hypertension 6:893-898, 1984)

KEY WORDS • natriuresis • acute hypertension • papillary plasma flow • renal papilla • papillary necrosis • bromoethylamine hydrobromide

The mechanism of natriuresis that accompanies acute elevation of renal perfusion pressure has been studied extensively; however it remains undetermined. Previous studies have indicated that acute increase in perfusion pressure is associated with an increase in intratubular pressure and a decrease in fractional sodium reabsorption in the proximal tubule.1,2 Others have found that sodium reabsorption in the distal tubule also is suppressed.3-5 Kunau et al.,6 however, demonstrated that fractional delivery of sodium to early and late distal tubules of superficial nephrons is not increased despite a twofold increase in urinary sodium excretion when renal perfusion pressure is increased from 120 to 170 mm Hg by epinephrine (E). These results provide evidence that juxtaglomerular nephrons or collecting ducts may play an important role in the mechanisms of pressure-induced natriuresis.

Recently, several studies have demonstrated a significant role of papillary plasma flow7 and papillary tissue tonicity* in the regulation of sodium excretion. The blood flow to the inner medulla of the kidney is poorly autoregulated, according to some investigators.8-9 "The inner medullary blood flow is increased linearly with acute elevation of renal perfusion pressure in the isolated perfused kidney, while cortical blood flow exhibits good autoregulation.10"

In the present study, we have examined the effect of acute increase in renal perfusion pressure on papillary plasma flow and its relationship to the sodium excretion. In addition, we have also examined the role of renal papillae in the regulation of sodium excretion following acute elevation of renal perfusion pressure.
Methods
Preparation of Rats
Studies were performed on male Sprague-Dawley rats. On the day of experiment, the rats were weighed and anesthetized with ether. The femoral artery was cannulated with a polyethylene tube (PEIO) for blood collection and blood pressure measurement; the left ventricle was also cannulated with a PEIO tube via the carotid artery for injection of microspheres. Renal blood flow (RBF) was measured with a microsphere method, as previously described. In addition, the jugular vein was cannulated with a PE50 tube for infusion purposes. A PE50 tube was inserted into the bladder for urine collection.

Measurement of Papillary Plasma Flow
Papillary plasma flow (PPF) was measured by a method originally described by Lilienfield et al. and modified by our laboratory. A midline abdominal incision was made and a loose nonocclusive umbilical tie was placed around the left renal pedicle. The tie was exteriorized through a small flank incision. The abdominal incision was sutured, and the animal was placed in a restraining cage. A solution of I\(^{125}\)-labeled albumin, 15 /uci/ml (Mallincrodt, St. Louis, Missouri), in 0.9% saline solution colored with 2% lissamine green dye was infused through the carotid catheter into the heart at a rate of 0.37 ml/min. The color of the lissamine green dye in the solution helped to identify the arrival of isotope into the carotid artery. One second before the isotope was infused into the heart, blood collection was begun from the femoral arterial catheter by free flow into a preweighed tube for exactly 20 seconds (approximately 0.08 ml), and the tie around the renal pedicle was pulled tight to occlude the RBF. Pentobarbital, 0.1 ml (60 mg/ml), was simultaneously infused intravenously at the time of ligation. The ligated kidney was removed immediately and frozen at — 20° C for at least 20 minutes. The papilla was weighed and counted together with the reference blood sample. The PPF was calculated according to the following formula:

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PPF (\text{ml/min/100 g papilla}) = \frac{\text{cpm/100 g papilla} \times 60 \text{ sec/min}}{\text{cpm/ml plasma} \times \text{perfusion time} \ (\text{sec})}
\]

The validity of this modified method in the measurement of PPF is based on three main reasons. 1) The isotope is adequately mixed inside the left ventricle, as we have previously determined by using radioactive microspheres. 2) The isotope reaches the femoral catheter and the kidney nearly at the same time, since the transit time of isotope from the left ventricle to the femoral catheter is less than 1 second in rats (determined in six rats, unpublished observations). 3) Our preliminary results confirmed the accuracy of the modified method. The average value of PPF (27.7 ± 1.31 ml/min/100 g, in 23 awake hydropenic rats) was similar to that obtained by others. Studies of the plasma volume distribution of I\(^{125}\) albumin in the renal papilla of 23 Sprague-Dawley rats over the time intervals of 10 to 30 seconds showed a linear distribution. Thus, the reference blood sampling could start before the infusion of I\(^{125}\) albumin. The details of the modified technique have been described by Bayle et al.

Clearance Studies
Inulin clearance was performed by a priming dose of inulin in normal saline, 200 mg/kg body weight (40 mg/ml solution), followed by a sustained infusion at a rate of 0.02 ml/min/100 g. Inulin clearance was started after 40 minutes of sustained infusion in each animal. At the middle of the urine collection, 0.6 ml of blood was obtained. The RBF and PPF were measured at the end of the clearance. Mean arterial pressure (MAP) was monitored throughout the experiments with a Gibson recorder and Statham transducer P23Db.

Experimental Groups
Experiments were performed in four groups of rats fed with Purina rat chow and surgically prepared as described above. Clearance studies were carried out without anesthesia.

Group 1
Animals weighing approximately 180 to 190 g were fasted overnight but allowed free access to water. Epinephrine (0.4 /u.g/min/100 g) mixed with sustaining inulin solution was infused throughout the clearance. Blood pressure usually stabilized after 20 minutes of E infusion. This dose of E had no effect on GFR, as shown previously by Kunau et al. Urine was then collected for 40 minutes for inulin and sodium determinations. Control animals were infused with sustaining inulin solution only. At the end of clearances, RBF and PPF were measured as described above.

Group 2
Papillary necrosis was induced in Sprague-Dawley rats weighing 180 to 190 g. A single dose of bromoethylyamine hydrobromide (BEA, 25 mg/100 g body weight), diluted in 0.5 ml of normal saline solution was infused into the rats through the tail vein. Development of papillary necrosis was virtually 100%, as documented in previous reports. Eight to 12 days after the BEA injection, animals were fasted overnight but allowed free access to water. The reason for performing the study 8 to 12 days after BEA injection was that if there were any toxic effects of BEA on the renal cortex they would have likely disappeared by this time. The experimental group was infused with 0.4 pig/min/100 g of E, and the control group was infused with an equivalent volume of saline. Clearance, RBF, and PPF measurements were performed as in Group 1. Papillary necrosis was evident in each animal, as the papillae had become grossly abnormal, yellowish, shrunk scar tissue.
Group 3

To achieve an identical reduction in GFR as in Group 2 animals with intact papillae, Sprague-Dawley rats weighing 180 to 190 g were subjected to bilateral cortical renal ablation through flank incisions under ether anesthesia. Partial nephrectomy was achieved by tightening ligatures around the upper and lower poles of both kidneys and excising the ischemic tissue distal to the ligature. Eight to 12 days after the surgery, the animals were fasted overnight and then underwent experiments identical to those in Group 2 rats. The RBF and PPF were not measured in this group of animals. The partially nephrectomized kidneys were examined, and the papillae were grossly intact in each animal.

Group 4

To examine the influence of papillary tonicity on sodium excretion after the acute elevation of renal perfusion pressure induced by E, Sprague-Dawley rats weighing approximately 150 g were subjected to chronic suppression of endogeneous antidiuretic hormone (ADH) release by the substitution of 5% dextrose solution for tap water for 2 weeks. The rats were then fasted overnight, but had free access to dextrose solution. After surgical preparation, clearance studies were performed by priming the animals with inulin and then administering a sustained infusion at a rate of 0.01 ml/min/100 g. A 30-minute urine collection was obtained; thereafter, E (0.4 µg/min/100 g) was infused intravenously. Clearance and PPF studies were carried out as in the previous groups. Another group of rats weighing approximately 180 g were fasted and dehydrated overnight. The clearance and PPF measurements before and after the E infusion were performed as described above. At the end of the studies, the right kidney was removed immediately for analysis of papillary tissue osmolality, as described by Appelboom et al.

Briefly, the papilla was rapidly dissected, weighed, and placed in a preweighed tube with 100 LC of distilled water. The tube was sealed tightly and immersed in 95° C water for 120 minutes. After the tissue was refrigerated for 24 hours, the tube was centrifuged, and the supernatant was removed for sodium, potassium, and urea determinations. The remaining tissue was dried in an oven for 24 hours to determine the amount of water content. The papillary tissue osmolality was estimated according to the formula: (Na + K) x 2 + urea.

Analytical Methods

Plasma and urine inulin concentrations were determined as previously described. Sodium was determined by flame photometer. Urinary osmolality was measured by freezing point depression. Urea concentration was determined by previously described methods.

The data were analyzed by unpaired or paired student's t test where appropriate, and all results were expressed as means ± SEM. Significance was set at the 5% level.

Results

Following E infusion, the MAP rose significantly; however RBF, GFR, and PPF were not different from those of control rats (Table 1, Group 1). Urinary sodium and fractional sodium excretions were increased, and urinary osmolality was decreased significantly after E treatment when compared to controls. In contrast, E infusion failed to increase sodium excretion in animals with papillary necrosis, despite a significant elevation of MAP (Table 1, Group 2). The GFR of the papillary necrosis rats decreased to less than half that of the normal rats, but it was not different between the E-treated and control animals. Papillary necrosis was associated with a very low PPF. The RBF increased slightly after E infusion, and urinary osmolality was not different between the E-treated and control rats (Table 1, Group 2).

After bilateral partial nephrectomy, the GFR was reduced by approximately 60% (Table 1, Group 3), and it was comparable to that of the papillary necrosis rats. Both absolute sodium excretion and fractional sodium excretion were not different between the control animals of Groups 2 and 3. In spite of the decreased GFR in animals with partial renal cortical ablation, sodium excretion was markedly increased following E infusion when compared to those of the control group (Table 1, Group 3). Urinary osmolality was not different between the control and E-infused rats.

Table 2 presents clearance data and the PPF of Group 4 animals before and after E infusion in hydrated and dehydrated rats. Before the E administration, urine flow rate was higher and urinary osmolality was lower in hydrated rats than those in dehydrated rats, respectively. The MAP and sodium excretion, however, were not different between hydrated and dehydrated rats. After E infusion, MAP, urine flow rate, and sodium excretion of both hydrated and dehydrated rats were greater than their respective values prior to the E infusion. Urinary osmolality decreased significantly after E treatment in dehydrated rats, whereas it was unchanged in hydrated animals. Medullary tonicity did not influence the renal handling of sodium excretion following acute elevation of renal perfusion pressure, since the sodium excretion was increased to the similar extent in both hydrated and dehydrated animals. In addition, PPF and papillary tissue osmolality also were not different between the hydrated and the dehydrated rats after E administration.

Discussion

The present observations confirm the previous findings that sodium excretion is increased when renal perfusion pressure is acutely raised by E administration in normal rats. The natriuresis was not associated with alterations of GFR and RBF. To define the role of renal papillae in the regulation of sodium excretion following the elevation of perfusion pressure, the effect of acute hypertension on the medulla was studied.
in rats with papillary necrosis induced by the administration of BEA. Infusion of E, however, failed to increase sodium excretion despite a marked increase of MAP. Failure to increase sodium excretion following acute elevation of perfusion pressure was not due to the decreased GFR, since sodium excretion was increased in bilateral partial cortical nephrectomy rats with comparable GFR. The results suggest that the renal papilla play a significant role in the mechanism of pressure-induced natriuresis.

Previous studies have suggested that PPF and renal medullary tonicity influence sodium excretion. An increase in PPF could decrease sodium transport in the loop of Henle, as it may reduce medullary interstitial hypertonicity. It is postulated that passive sodium transport occurs along the thin ascending limb of Henle as a consequence of a sodium concentration gradient between the medullary interstitium and the tubular lumen. Greater sodium concentration in the lumen of ascending limb that is in the medullary interstitium is established by water extraction from the descending limb of Henle by the hypertonic medulla. Thus increased PPF may lead to washout of the interstitium is established by water extraction from the descending limb of Henle by the hypertonic medulla.
Henle. The important role of PPF in the regulation of sodium excretion is further supported by a recent study demonstrating that increased sodium excretion after administration of acetylcholine and bradykinin. Our present study, however, failed to demonstrate any association between increased sodium excretion and alteration of medullary blood flow after acute elevation of renal perfusion pressure. The PPF was unchanged despite a significant rise in MAP.

There are two anatomically different nephron populations in the renal cortex. One is the superficial nephron, which consists of a short loop of Henle traversing through the outer medulla. The other is the juxtamedullary nephron, which has a long loop of Henle descending into the inner medulla and bending at the tip of papilla. Papillary necrosis, therefore, presumably will eliminate the renal function of juxtamedullary nephrons and collecting ducts. Hence, failure to increase sodium excretion after acute elevation of renal perfusion pressure in animals with papillary necrosis would suggest that the tubular segments of the juxtamedullary nephron or the collecting ducts are primarily responsible for the natriuresis following the E infusion. Kunau et al. have also demonstrated that fractional delivery of sodium to early and late distal tubules of the superficial nephron is not increased despite a twofold increase in urinary sodium excretion when renal perfusion pressure is elevated by E.

Our conclusion, however, is valid only when superficial nephrons of BEA-treated animals are functionally intact. The latter is probably true, since micropuncture studies have indicated that the single nephron GFR of superficial nephrons of BEA rats is not different from that of control rats despite a 40% reduction of whole kidney GFR 18 to 24 hours after BEA injection. In addition, the concentration ratio of distal tubular fluid inulin to plasma inulin in the superficial nephrons is not different between the control and BEA-treated animals. There is no leakage of inulin through the collecting ducts. Thus, there is no evidence of tubular necrosis or functional abnormalities of superficial nephrons in BEA-treated rats.

Other investigators have indicated that the elevation of perfusion pressure is associated with decreased sodium transport in the proximal tubule of the superficial nephrons. This finding does not necessarily contradict our results. However, since rats with papillary necrosis failed to increase sodium excretion after acute elevation of perfusion pressure, one has to assume that the increased sodium delivery from the proximal tubule of the superficial nephron is completely recaptured by the distal nephron. Micropuncture studies of deep nephrons have not been performed in rats following acute elevation of perfusion pressure. It is not certain, therefore, whether the response to acute hypertension is similar between the proximal tubules of the superficial nephrons and the proximal tubules of the juxtamedullary nephrons.

Sodium reabsorption in the distal tubule is suppressed after acute increase in perfusion pressure. Renal clearance studies by Bank et al. have shown that acute elevation of the perfusion pressure results in a decrease of both free water formation (CH₂O) and reabsorption (TcH₂O). Furthermore, it is demonstrated that decreased TcH₂O is associated with reduced papillary sodium content. We have also found that urinary osmolality was decreased after acute elevation of renal perfusion pressure. These findings suggest that inhibition of sodium transport in the distal nephrons of the juxtamedullary glomeruli is primarily responsible for the pressure-induced natriuresis.

Finally, E tends to decrease sodium excretion in isolated rat kidney perfused at a constant pressure. The fact that the dose of E used in this study is, of itself, not natriuretic is supported by the previous observations. There is no appreciable increase in sodium excretion when E (0.4–1.7 /mg/min) is infused in rats and the renal perfusion pressure is kept at a constant level by aortic constriction.

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References

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