Rat Renal Interstitial Bradykinin, Prostaglandin $E_2$, and Cyclic Guanosine $3',5'$-Monophosphate
Effects of Altered Sodium Intake
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Abstract
Kinin generated intrarenally probably affect renal function by altering levels of various mediators and messengers, including prostaglandin $E_2$ ($PGE_2$) and cyclic guanosine $3',5'$-monophosphate (cGMP). Using a microdialysis technique, we monitored levels of cortical and medullary renal interstitial fluid kinins, $PGE_2$, and cGMP after 5 days of 0.15% (low), 0.28% (normal), or 4.0% (high) sodium intake. Samples were collected from anesthetized rats ($n=5$ for each diet). During normal sodium intake, renal interstitial fluid kinin, $PGE_2$, and cGMP levels in dialysate leaving the cortex were $113.0\pm 5.8$ pg/min, $1.23\pm 0.11$ pg/min, and $0.03\pm 0.004$ pmol/min, respectively. In the fluid leaving the medulla, the levels were $93.0\pm 17$ pg/min, $2.28\pm 0.14$ pg/min, and $0.08\pm 0.005$ pmol/min, respectively. In rats consuming a low sodium diet, renal cortical interstitial fluid kinin and cortical and medullary $PGE_2$ and cGMP appearance rates were significantly increased ($P<.01$). Rats consuming a high sodium diet showed renal cortical and medullary kinin levels that were decreased 100-fold ($P<.01$), whereas $PGE_2$ and cGMP were increased ($P<.01$), compared with levels in rats consuming normal sodium intake. Renal interstitial fluid kinin is extremely sensitive to dietary sodium, but changes in interstitial fluid $PGE_2$ and cGMP are not always directionally similar, suggesting different regulations of these substances in response to sodium intake.

Methods
Renal Microdialysis Technique
For the determination of renal interstitial fluid kinin, $PGE_2$, and cGMP, we constructed a microdialysis probe as previously described. Two 30-cm-long, hollow polyethylene tubes (0.12-mm inner diameter, 0.65-mm outer diameter; Bioanalytical Systems) were inserted into each end of a 1-cm-long hollow fiber dialysis tubing (0.3-mm inner diameter; molecular mass cutoff, 5000 D; Hospal) adjusted so that the distance between the ends of the polyethylene tubes was 3 mm (dialysis area) and were sealed in place within the dialysis tubing with cyanoacrylate glue. The dead volume of the dialysis tubing and outflow tube was $2.3 \mu L$. The known kinin-, eicosanoid-, and NO-generating and -degrading enzymes (MW, 34 000 to 150 000) will not cross the dialysis membrane because of their size. Then the microdialysis probe was sterilized by a gas sterilization method.

Animal Preparation and Renal Interstitial Fluid Collection
Experiments were conducted in three groups of 6-week-old female Sprague-Dawley rats ($n=5$ each) purchased from Harlan Sprague Dawley Inc. After arrival at the age of 5 weeks, the groups consumed a diet containing either 0.15% (low), 0.28% (normal), or 4.0% (high) NaCl for 5 consecutive days. Then with the rats under general anesthesia with $80 \text{mg/kg}$ ketamine IM (Parke-Davis) and $8 \text{mg/kg}$ xylazine IM (Mobay Corp), the right kidney was exposed via a midline abdominal incision. The right renal capsule was penetrated with a 31-gauge needle that was tunneled in the outer renal cortex approximately 1 mm from the outer renal surface for 0.5 cm before it exited by penetrating the capsule again. Then one end of the dialysis probe was pulled through until the dialysis fiber was situated in the renal cortex. Next, the needle was pulled out. The same procedure was repeated to place the dialysis probe into the right renal medulla, except the dialysis fiber was placed approximately 5 mm from the outer renal surface. The inflow tubes of the renal interstitial cortical and medullary dialysis...
Fig 1. Bar graph of renal interstitial fluid bradykinin level of anesthetized rats consuming a 0.15% (low), 0.28% (normal), or 4.0% (high) sodium diet (n=5 for each diet). Hatched bars indicate renal cortex; solid bars, renal medulla. *P<.01 compared with normal diet; +P<.01 compared with corresponding medulla.

probes were connected to gas-tight syringes filled with lactated Ringer's solution and perfused at 3 μL/min (pump 22, Harvard Apparatus). At this perfusion rate in vitro recovery was 78% for bradykinin and 70% for cGMP. A 90-minute recovery period elapsed before the experimental protocol was initiated. The effluent was collected from the outflow tube for 270 minutes. For kinin measurements effluent was collected in nonheparinized plastic tubes containing 0.1 mL ethanol. The effluent for PGE2 and cGMP measurements was collected in plastic tubes on ice. The renal interstitial samples were stored at -20°C until they were assayed.

Analytical Methods
Renal interstitial fluid kinin, PGE2, and cGMP levels in dialysate samples were measured by radioimmunoassays.12-14

Statistical Analysis
Data were examined by analysis of variance, including a repeated-measures term, using the general linear model procedures of the Statistical Analysis System Institute.15 Values are given as mean±SEM. Statistical significance was identified at a level of P<.05.

Results
Renal Interstitial Kinin
Kinin measured by radioimmunoassay in dialysate from the rats consuming a normal sodium diet (0.28%) appeared at a rate of 113±8 pg/min from the cortex and 93±17 pg/min from the medulla (Fig 1). In animals consuming a low sodium diet (0.15%), interstitial fluid kinin appearance rate was significantly greater (316±36 pg/min, P<.01) from the renal cortex but not from the medulla (112±37 pg/min, P=NS). In rats consuming a high sodium diet (4%), both cortical and medullary kinin appearance rates were markedly decreased to 0.9±0.4 and 0.5±0.1 pg/min (each P<.01).

Renal Interstitial PGE2
As shown in Fig 2, rats consuming a normal sodium diet elaborated PGE2 at a rate of 1.23±0.11 pg/min from the cortex, significantly less than that from the medulla (2.28±0.14 pg/min, P<.01). These levels were each significantly greater in rats consuming a low sodium diet (3.12±0.25 and 3.33±0.22 pg/min, respectively; each P<.01). In contrast to the kinin levels in the same animals consuming a high sodium diet, PGE2 appearance rates were increased (3.58±0.56 and 4.48±0.38 pg/min for the cortex and medulla, respectively; each P<.01 compared with a normal diet).

Renal Interstitial cGMP
Renal cGMP appearance rates were 0.05±0.004 pmol/min from the cortex and 0.08±0.005 pmol/min from the medulla of rats consuming a normal sodium diet (Fig 3). The low sodium diet was associated with cGMP appearance rates in both cortical and medullary dialysate that increased significantly to 0.40±0.1 and 0.30±0.1 pmol/min, respectively (each P<.01). Like the PGE2 appearance rates, there were increased cortical and medullary cGMP levels in rats consuming a high sodium diet (0.20±0.06 and 0.14±0.01 pmol/min, respectively; each P<.01).

Discussion
Renal kallikrein and kinin play a role in the regulation of renal electrolyte and water homeostasis. Neither the mechanisms by which this system exerts such control nor the specific functional responsibilities are fully understood. However, the localization and regulation of various components of the system by renal proteases and peptidases support the hypothesis that kinins act to modulate renal function through effects on renal vascular resistance and perhaps tubular ion and water transport. Some of these effects are believed to be mediated through release of eicosanoids or endothelium-derived relaxing factor (NO).6,7 Because there is a correlation between NO-induced vasodilation and NO-induced cGMP accumulation in vascular smooth muscle cells,
cGMP accumulation is a useful tool to use to estimate the release of NO acting on adjacent cells and tissue. For many years urinary kallikrein excretion was the only variable of the renal kallikrein-kinin system available for measurement, and in situ renal activity of the system was inferred from this determination. Now the activity of this system is beginning to be assessed by measurement of levels of urinary kinins, kininases, and kininogens. However, the relation of the kinin level, even in ureteral urine, to upstream activity at kinin receptor sites is uncertain. The complete extent of renal kininase activities in vivo has yet to be defined, and their regulation is unclear. The contribution to renal kinin production by extrinsically produced (hepatic) kininogens versus kininogen synthesized in the renal tubule is unknown. In the present study we obtained a measurement of system activity closer to the presumed sites of kinin action on renal vasculature and renal interstitial and epithelial cells. In addition, these measurements can be made in time periods that will allow future evaluations of any immediate changes in the system activity in relation to renal hemodynamics and excretory function and in response to various stimuli or maneuvers.

We monitored changes in renal interstitial fluid kinin, PGE₂, and cGMP levels in response to changes in dietary sodium intake. During normal sodium intake we were able to measure basal renal cortical and medullary interstitial fluid kinin, PGE₂, and cGMP levels. PGE₂ was significantly higher in the renal medulla than in the cortex, indicating its major site of production under normal conditions. Renal cortical interstitial fluid kinin level was 2.8-fold higher in rats consuming a low sodium diet than in rats consuming a normal sodium diet, supporting previous observations. However, rats consuming a high sodium diet had renal interstitial fluid kinin levels that were reduced 300-fold less than levels in animals consuming a low sodium diet. This result shows an unexpected and extreme sensitivity of renal interstitial fluid kinin to changes in dietary sodium. In contrast, interstitial fluid PGE₂ and cGMP levels in cortex and medulla were increased in animals consuming a low or high sodium diet. Higher levels of PGE₂ and cGMP in the rats consuming a low sodium diet may be related to the increase in kinin, but the high levels seen during high sodium intake might reflect alternate regulation of these substances. It is unlikely that renal interstitial volume would significantly change in response to different sodium intakes and influence recovery rates of kinin, PGE₂, and cGMP. If in the present study there was any expansion of renal interstitial volume during high sodium intake, it would simultaneously reduce kinin concentration and increase PGE₂ concentration—a possible but unlikely result. It is also possible that trauma and hemorrhage around the microdialysis probe activate plasma prekallikrein and thus are responsible for kinin generation from plasma kininogens, although no hemorrhage was observed during probe insertion in the renal tissue. Furthermore, renal interstitial kinin levels were very low during high sodium intake, which is similar to our previous observations in the chronically instrumented conscious dog. In a previous study, kinin receptor blockade in dogs decreased urine flow rate and sodium excretion during low sodium but not during high sodium intake. This result may be related to the present observations, ie, that high sodium diet is associated with barely detectable renal kinin levels, and thus there is minimal influence of such levels on parameters of renal function. It is still unclear why an increase in the production of renal kallikrein and kinins, both natriuretic substances, occurs in response to a reduction in dietary sodium intake. The extremely high intrarenal kinin levels that occur in response to low sodium intake suggest that this system functions to counteract vasoconstrictor and anti-diuretic mechanisms activated in response to sodium depletion. Regardless, the marked differences in intrarenal kinin levels in the present study suggest that important information about the role(s) of the renal kallikrein-kinin system can be obtained with this technique.

Acknowledgments
This work was supported by grant HL-476629 from the National Heart, Lung, and Blood Institute, National Institutes of Health, to H.M.S. Dr Helmy M. Siragy is the recipient of a Research Career Development Award (1 K04 HL-03006) from the National Institutes of Health, Bethesda, Md.

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_Hypertension_. 1994;23:1068-1070
doi: 10.1161/01.HYP.23.6.1068

_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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