Effect of Increased Perfusion Pressure on Proximal Tubular Reabsorption in the Isolated Kidney[1] (37423)

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In a recent study reported from this laboratory (1), it was demonstrated that increasing renal arterial pressure in the isolated dog kidney caused a significant natriuresis in the face of a constant glomerular filtration rate and renal blood flow. On the basis of a slight but significant increase in solute-free water reabsorption (TcH_2O), we inferred that sodium reabsorption was decreased along the proximal tubule.

In the present experiments, we sought to define the mechanism of pressure natriuresis in this preparation by more direct methods. Accordingly, micropuncture techniques were employed to examine the effect of increasing renal perfusion pressure on proximal tubule sodium reabsorption and gromerular filtration rate of superficial cortical nephrons.

Methods. All studies were performed on mongrel dogs weighing between 15 and 25 kg fed a standard kennel ration. On the day prior to the study, the animals were deprived of food but water was permitted *ad libitum.* On the morning of the study, the animals were anesthetized with sodium pentobarbital, 30 mg/kg, administered intravenously with supplemental doses as required. An endotracheal tube was inserted and respirations were regulated with a Harvard respirator adjusted to maintain arterial pH between 7.35 and 7.45.

The preparation of the isolated kidney was similar to that previously described (1, 2). After perfusion of the isolated kidney was established, the perfusion animal received a priming dose of inulin followed by a constant infusion of inulin in 0.9% NaCl at 1.0 ml/min; aqueous Pitressin was added to the infusion to deliver 0.5 mU/kg/min. A minimum of 60 min was allowed for equilibration between blood and perfusate and stabilization of renal function. In all experiments, collection of urine samples began within 90 min of the time perfusion of the isolated kidney was started.

Group I consisted of 10 experiments. At the conclusion of the equilibration period one or two consecutive 30-min control urine samples with midpoint arterial blood samples were collected. Then renal arterial pressure (P_{RA}) was increased approximately 50 mm Hg by raising the perfusion dog platform. After a 15-min stabilization period, either one or two 30-min experimental urine samples were collected.

Group II consisted of 11 experiments in which the function of the isolated kidney was examined over the same time interval as in Group I but P_{RA} was maintained constant throughout the study.

In the control and experimental periods of each group, single nephron glomerular filtration rate (SNGFR) and fractional reabsorption were measured in proximal tubules of superficial cortical nephrons. After decapsulation of a 2-cm^2 area of cortex, end proximal tubule segments were identified by the injection of 0.2–0.3 ml of a 5% aqueous solution of lissamine green into the arterial cannula of the isolated kidney. Segments so identified were punctured with sharpened glass capillary micropipettes of 8–12 μm tip size. A small droplet of sudan black stained mineral oil was injected to ascertain direction of flow and to verify location in the last surface seg-

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ment. A column of oil, four to six tubule diameters in length, was injected distal to the micropipette tip. An accurately timed collection (30–60 sec) of tubular fluid was made by gentle hand aspiration to keep the distal oil block stationary and the tubule diameter relatively constant. At the end of the collection the micropipette tip was sealed with oil to prevent sample evaporation. To avoid the possible artifactual problem of recollection micropuncture technique in the dog (3) as well as the technical problems related to performing recollection micropuncture in a heparinized kidney, different end-proximal tubule segments were punctured in control and experimental periods. Proximal tubule pressure was measured in Groups I and II as previously described (1). Five or six micropuncture samples and proximal tubule pressure determinations were obtained from previously unpunctured nephrons in each period and averaged to give a mean value for that period.

Blood pressure monitoring and recording, urine collection and blood sampling were carried out as previously described (1). All blood and urine samples were analyzed for sodium and inulin; packed cell volume (PCV) and plasma protein concentration were determined on all blood samples. Sodium was measured with an Instrumentation Laboratories flame photometer. Inulin was measured by the method of Fuhr, Kaczmarczyk, and Kruttgen (4) and plasma protein concentration by refractometry (Goldberg refractometer, American Optical Company, Buffalo, N.Y.). PCV was determined using a microhematocrit centrifuge. Renal blood flow (RBF) was measured directly by timing the flow from the renal vein into a graduated cylinder. Filtration fraction (FF) was determined from the formula FF = Cin/RPF, where Cin is the inulin clearance and RPF is the renal plasma flow calculated according to the formula RPF = RBF (1–0.95 PCV). Tubule fluid sample volume was measured with a calibrated micropipette; tubular fluid inulin concentration was measured in duplicate by means of the fluorometric method (5). SNGFR was calculated according to the formula SNGFR = $V_{TF} \times$ (TF/P)in, where V_{TF} is the tubular fluid flow rate in nl/min and (TF/P)in is the tubular fluid to plasma inulin ratio. Proximal fractional reabsorption equals 1 — (P/TF)in $\times$ 100%. Proximal absolute reabsorption equals SNGFR — V.

The data in the text and tables are expressed as the mean ± SE. Student's t test was used for statistical analysis of paired data within each group and mean data between groups (6).

Results. The data from Groups I and II are summarized in Table I.

In Group I, increasing P_{RA} from 103 ± 1 to 155 ± 2 mm Hg resulted in an increase in $U_{Na}V$ from 40.9 ± 10.2 to 94.6 ± 16.0 μequiv/min, $p < 0.01$. Cin and plasma protein concentration did not change whereas RPF and PCV decreased and FF increased significantly. In Group I (TF/P)in decreased in 7 and increased in 3 experiments. However, only in 2 experiments was the decrease greater than 10% of the control (TF/P)in so that the mean change for the group was not statistically significant. Thus, proximal tubular fractional reabsorption measured 46 ± 3 in the control period and 45 ± 3% during the period of elevated renal arterial pressure. Since no significant change in SNGFR was detected, these data provide no evidence to indicate either that proximal tubule absolute sodium reabsorption was decreased or that the amount of filtrate delivered out of the proximal tubule (V_{TF}) was increased. The ratio of SNGFR/Cin, an indicator of filtrate distribution, remained unchanged. The twofold increase in proximal tubule pressure was significant, $p < 0.005$.

In Group II (Table I), function of the isolated kidney with P_{RA} held constant was examined over the same time interval as in Group I. In the absence of a pressure stimulus, sodium excretion in the isolated kidney did not change. Cin, SNGFR, (TF/P)in and P_{PT} also did not change significantly. Similar to Group I, RPF decreased.

Discussion. The purpose of this study was to define the mechanism(s) of pressure natriuresis in the isolated kidney using micropuncture techniques.

Similar to our previous report (1) we observed that increasing P_{RA} of the isolated kidney resulted in a significant increase in sodium excretion that could not be explained by changes in GFR, RBF, PCV or plasma

TABLE I. Summary of Data from the Isolated Kidney in Groups I and II During Control (C) and Experimental (E) Periods.[a]

		$U_{Na}V$ (μEq/min)	Cin (ml/min)	RPF (ml/min)	FF	PCV (%)	Plasma protein (g %)	P_{RA} (mm Hg)	(TF/P)in	SNGFR (nl/min)	SNGFR/ Cin ($\times 10^{-6}$)	V_{TF} (nl/min)	P_{PT} (mm Hg)
Group I ($N = 10$)	C	40.9 ±10.2	37.5 ±3.2	114 ±9	0.34 ±0.03	39 ±1	4.3 ±0.1	103 ±1	1.85 ±0.11	99 ±12	2.67 ±0.30	54.6 ±6.4	13.3 ±1.0
	E	94.6[c] ±16.0	37.7 ±3.7	98[c] ±9	0.41[b] ±0.05	38[b] ±1	4.1 ±0.2	155[d] ±2	1.81 ±0.12	98 ±11	2.68 ±0.32	55.0 ±6.3	25.3[c] ±2.9
Group II ($N = 11$)	C	40.3 ±11.7	32.4 ±2.4	107 ±5	0.30 ±0.02	36 ±2	4.6 ±0.1	105 ±1	1.99 ±0.10	100 ± 8	3.23 ±0.29	51.2 ±4.2	11.5 ±1.4
	E	34.1 ±13.8	30.5 ±2.5	90[c] ±3	0.34 ±0.02	35[b] ±2	4.4 ±0.2	106 ±1	1.94 ±0.08	95 ± 7	3.26 ±0.29	49.4 ±3.8	10.8 ±1.1

[a] Data are presented as mean ± SE.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

protein concentration. Measurements of SN GFR and (TF/P)in ratio in superficial nephrons permitted us to evaluate the contribution of the proximal tubule to the natriuresis. No significant changes in either SNGFR or (TF/P)in were observed in response to elevating P_{RA}. These findings suggest that absolute tubule sodium reabsorption along the accessible portion of the proximal tubule was not depressed during the pressure stimulus. In addition, since SNGFR did not change, these data provide no evidence that the volume of filtrate delivered out of the proximal tubule was augmented. Thus, these results are consistent with the interpretation that pressure natriuresis in the isolated kidney derives from a decrease in sodium reabsorption at some site distal to the accessible portion of the proximal tubule.

The absence of a significant decrease in proximal (TF/P)in in response to a rise in P_{RA} is in agreement with the observations of Stumpe, Lowitz and Ochwadt (7) in the chronically hypertensive rat but at variance with the findings of Koch, Aynedjian, and Bank (8) and Dresser, Schneider, Lynch and Knox (9) in the acutely hypertensive rat and dog, respectively. The latter two groups of investigators found that acutely raising renal arterial pressure caused a significant fall in proximal tubule fractional sodium reabsorption although a natriuresis occurred only in the study of Koch *et al.*

At least two possibilities may explain the difference between our results and those of the above investigators. First, it is possible that raising P_{RA} does depress proximal tubule fractional sodium reabsorption but that under the conditions of our experiments the rise in FF resulted in an increase in postglomerular capillary plasma colloid osmotic pressure which acted as an equal but opposing force to the pressure stimulus so that no change in proximal tubule (TF/P)in was detected. This explanation most likely accounts for the apparent discrepancy between the present experiments and our earlier study (1) in which we observed a slight but statistically significant increase in TcH_2O which we interpreted to mean that proximal tubule sodium reabsorption was decreased. In our earlier study RBF and FF did not change.

The reason for the change in RBF and FF in the present experiments is not apparent.

A second explanation may be that the technique of puncturing random end-proximal tubules is insufficiently sensitive to detect small changes in (TF/P)in. This possibility cannot be excluded, although it should be noted that using this technique, we were able to detect changes in proximal (TF/P)in in the isolated dog kidney following saline loading (10) similar to those observed by other investigators employing recollection micropuncture in the intact dog kidney (3, 11–13). Even if it is assumed that a small change in (TF/P)in escaped detection, it is still necessary to postulate that elevating renal perfusion pressure altered the handling of sodium by some more distal nephron segment in order to account for the natriuresis. Several groups of investigators have shown that decreases in proximal tubule sodium reabsorption and increases in distal sodium delivery do not result in a significant natriuresis unless distal tubule sodium transport is also depressed (13–17).

Other investigators employing a variety of experimental techniques have also concluded that distal tubule sodium transport is altered by an increase in renal arterial pressure. Clearance data obtained from studies in man (18), rat (19), and dog (20–22) and micropuncture data from studies in the rat (7) provide support for the concept that transport along the loop of Henle is depressed when P_{RA} is elevated. Tobian *et al.* (23), using the stop-flow technique, found that increasing P_{RA} impairs the ability of the distal nephron to achieve a minimum sodium concentration.

In agreement with our earlier findings (1), we observed a significant rise in P_{PT} when P_{RA} was increased. The measurement of SN GFR and (TF/P)in permitted us to evaluate the genesis of the rise in P_{PT}. In our initial report, we presented a theoretical argument against the possibility that the rise in P_{PT} reflected an increase in SNGFR secondary to a redistribution of filtrate from deep to superficial nephrons. The constancy of SN GFR and SNGFR/Cin in the present study supports our theoretical argument. We also considered the possibility that the rise in P_{PT} reflected an increase in proximal tubule fluid flow rate secondary to a decrease in proximal tubule absolute sodium reabsorption; but in the absence of a significant change in (TF/P) in this appears unlikely. Thus, from our data the rise in P_{PT} seems best explained by an increase in tubule fluid flow rate at some point beyond the accessible portion of the proximal tubule. In view of the findings of other investigators it seems likely that the increase in P_{PT} reflects a decrease in sodium and water reabsorption along the loop of Henle.

Summary. The present experiments demonstrate that raising renal perfusion pressure in the isolated kidney caused a significant increase in sodium excretion in the absence of detectable changes in proximal tubule sodium transport suggesting that the natriuresis resulted primarily from a decrease in sodium transport along more distal nephron segments. The constancy of SNGFR/Cin argues against a role for redistribution of glomerular filtrate in the natriuresis.

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