The Effect of Altered Sodium Concentration in the Distal Nephron Segments on Renin Release

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ABSTRACT Ethacrynic acid, a potent inhibitor of sodium reabsorption in the ascending limb of Henle's loop, produces a sharp rise in renal venous renin activity within 5 min after intravenous administration in anesthetized dogs. This response persists when volume depletion is prevented by returning urinary outflow to the femoral vein. Comparable studies with chlorothiazide, a diuretic with little or no effect on the medullary portion of the ascending limb of the loop of Henle, failed to produce a significant increase in renal venous renin activity.

When administered during ureteral occlusion, ethacrynic acid produced no change in renal venous renin activity until ureteral occlusion was released and flow restored. Following release of the ureters, a prompt rise in renal venous renin was again observed within 5 min of release. Control studies of ureteral occlusion yielded a fall in renal venous renin activity following release of the ureter without administration of ethacrynic acid. These studies identify a prompt stimulatory effect of ethacrynic acid on renin release that is unrelated to volume depletion but dependent upon the presence of tubular urine flow. Although further definition of the site and characteristics of the distal tubular mechanism for stimulation of renin release requires more direct study, the data presented here indicate that changes in sodium concentration in distal tubular fluid serve as a stimulus for renin release.

INTRODUCTION

Although the importance of the renin-angiotensin system as a regulatory mechanism for sodium reabsorption

has been clearly established, the factors that influence the rate of renin release are still poorly defined. Various hemodynamic parameters (1–3), particularly the renal arterial perfusion pressure (2, 3), have been shown to play a significant role. There is also some evidence that sodium balance influences renin release (4–6). In addition, several studies have recently suggested that the mechanism controlling the release of renin is responsive to changes in tubular fluid sodium (7–10). However, the data from these studies have appeared to be contradictory in some aspects. On the basis of micropuncture data, Thurau (7) and Thurau, Schnermann, Nagel, Horster, and Wahl (8) have proposed that tubular fluid sodium in the macula densa segment of the distal convolutions may function as a stimulus for a feedback mechanism that alters glomerular filtration in individual nephrons. When their experiments were performed on the renin-rich kidneys of sodium-depleted rats, these investigators noted that retrograde microinjections of sodium chloride (150 or 300 mmoles/liter) into the distal convolutions were promptly followed by collapse of the proximal segment of the distally perfused nephron; proximal and distal segments of the same nephron were identified by proximal injections of Lissamine green. This proximal collapse was interpreted as evidence of decreased filtration due to vasoconstriction of the afferent arteriole. Since this response was less consistently observed when comparable studies were performed on the renin-poor kidneys of unilaterally nephrectomized, sodium-loaded rats, it was also concluded that high sodium concentrations in the macula densa segment may function as a stimulus for the release of renin, which then mediates the effect on the afferent arteriole.

This interpretation of these data was recently questioned by Gottschalk and Leyssac (11) who attributed the finding of proximal collapse after distal saline injections, in similar micropuncture studies, to the leakage of fluid from the proximal puncture sites used for

earlier injections of Lissamine green. They also were unable to reproduce the phenomenon of proximal collapse when the studies were performed on nondecapsulated kidneys. However, the relevance of these observations remains as yet undetermined, since proximal collapse was observed by Thurau even in studies in which the proximal segments were not previously punctured (11).

A mechanism linking the vascular resistance in the afferent arterioles with the solute concentration in the distal convoluted tubules also was postulated by Guyton, Langston, and Navar (12, 13), although their data would suggest that increased osmolality in the distal convolutions may cause vasodilatation, rather than vasoconstriction, of the afferent arterioles. Furthermore, an intrarenal mechanism relating the rate of renin release to tubular fluid sodium was suggested by the studies of Vander and Miller (9). In these studies, on the other hand, acetazolamide, chlorothiazide, and osmotic diuretics, which should have increased the quantity of sodium delivered to the distal nephron segments, appeared to inhibit the renin response to aortic constriction. The most effective inhibitors of renin release, however, were the osmotic diuretics, and although the delivery of sodium to the macula densa segment is increased by these agents, the sodium concentration in the distal convolutions is unaltered or decreased (14–16). Also, it is possible that volume expansion could account for the failure of renin to increase during aortic constriction when these agents were used.

To test the hypothesis that high sodium concentrations in the macula densa segment of the distal convolutions may stimulate an increase in renin release, we have studied the effect of ethacrynic acid, which inhibits sodium reabsorption in the ascending limb of the loop of Henle (17–19), on renal venous renin activity (RVRA) in anesthetized dogs. In one group of experiments, the effect of ethacrynic acid was compared with the effect of chlorothiazide, a less potent diuretic which has less effect proximal to the postulated "sensor" in the macula densa segment than does ethacrynic acid (20, 21). Volume depletion during the period of natriuresis was prevented by shunting the urine into the femoral veins. In additional experiments, the effect of ethacrynic acid on RVRA was shown to be dependent upon tubular flow.

METHODS

Experiments were performed on 26 adult female mongrel dogs ranging in weight from 15.2 to 26.7 kg. The animals were lightly anesthetized with pentobarbital and ventilated through an endotracheal tube attached to a Harvard respirator (Harvard Apparatus Co., Millis, Mass.). The left renal vein was exposed through a mid-line abdominal incision and cannulated via the ovarian vein with a small polyethylene catheter. Constant infusion of a solution of

0.9% sodium chloride through the renal vein catheter at a rate of less than 0.4 ml/min prevented clotting. Bleeding was carefully controlled and blood flow through the renal vein was not interrupted. Both ureters were then exposed just proximal to the bladder and catheters were inserted to a level near the renal pelvis. The abdominal incision was closed and control urine samples were collected during a period of approximately 30 min. During the second half of this control period three blood samples were collected from the renal vein catheter in iced tubes containing small amounts of EDTA for determinations of RVRA. All blood removed for these and subsequent determinations of RVRA was replaced with blood obtained from donor dogs before each experiment. After the initial control period, the experimental protocol was different for each of four groups of animals.

The concentration of sodium in urine was determined by flame photometry. Samples of renal venous blood were prepared for bioassay by the method of Helmer (22). The pressor response of the rat to angiotensin formed during incubation of the plasma was measured as an index of renin activity as described by Higgins, Davis, Urguhart, and Olichney (23).

Free urine flow studies. Ethacrynic acid (50 mg) was given as a single intravenous injection and, in studies performed on four animals, urine was collected from both ureters for measurements of sodium excretion during 5-min periods extending through the next 20 or 30 min, followed by longer collection periods of from 10 to 30 min duration, for a total of either 1 or 2 hr. Samples of renal venous blood were obtained for determinations of RVRA at the end of each collection period.

Reinfusion studies. Following the administration of ethacrynic acid (50 mg), the ureteral catheters of eight animals were inserted into the femoral veins and the urine that was formed in the period of diuresis was shunted directly into the vena cava. Samples of blood were obtained from the renal vein catheters as in the free urine flow studies. At the end of each experiment, the ureteral catheters were removed from the femoral veins and urine was collected to determine the rate of urine flow and sodium excretion from each kidney. In comparable studies on six animals, samples of renal venous blood were obtained for determinations of RVRA before and after the intravenous administration of chlorothiazide (500 mg).

Ureteral occlusion studies with ethacrynic acid. At the end of the control period, in studies on four animals, the pressure in the renal pelvis of each kidney was abruptly raised to 65 mm Hg by the retrograde injection of bladder urine through the ureteral catheters, which were then occluded with rubber-shod clamps. With the ureteral catheters occluded, three blood samples for determinations of RVRA were collected from the renal vein catheter at 5-min intervals. Ethacrynic acid (50 mg) was then injected intravenously and three additional samples of renal venous blood were collected 5 min apart. Following this, the clamps on the ureteral catheters were released and urine was collected serially from each kidney in 1.0 ml aliquots. Samples of renal venous blood were obtained 5, 10, and 15 min after the restoration of urine flow.

Ureteral occlusion studies without ethacrynic acid. The ureteral catheters of four animals were occluded after urine flow was stopped as in the ureteral occlusion studies with ethacrynic acid. Three samples of renal venous blood were obtained 5 min apart with the ureters occluded. The clamps on the ureters were then released, and renal venous blood

TABLE I

Urine Flow Rate, Sodium Excretion, and RVRA before and after the Administration of Ethacrynic Acid in a Typical Free Urine Flow Experiment

Time	Right kidney			Left kidney	
	Urine flow	$U_{Na}V$	RVRA*	Urine flow	$U_{Na}V$
min	*ml/min*	*μEq/min*		*ml/min*	*μEq/min*
			475		
			650		
0–30	0.1	10.1	450	0.1	17.8
Ethacrynic acid 50 mg administered intravenously					
30–35	1.8	339.0	1650	1.7	325.4
35–40	4.7	648.7	1750	4.7	661.3
40–45	4.6	652.7	2000	4.3	610.2
45–50	4.2	579.1	2000	3.8	515.3
50–60	3.8	520.5	2200	3.4	443.8
60–70	3.5	451.5	1500	3.0	396.9
70–80	3.0	387.0	1650	2.4	302.4
80–90	2.4	322.5	1300	1.9	236.6
90–120	1.5	184.3	2000	1.0	113.1
120–150	0.7	72.0	1000	0.4	45.7

* Nanograms of angiotensin II formed per 100 ml of plasma.

samples were obtained 5, 10, and 15 minutes after urine flow was restored.

Statistical methods. For statistical comparisons of the data on RVRA, the values for the samples collected at 5-min intervals, for example, S_1, S_2, and S_3 from the control period, were averaged ($S_1 + S_2 + S_3/3 = S_A$) and this value, S_A was used in calculating the means and standard errors for each group of experiments with n = the number of experiments in each group. For intra-group comparisons, Student's *t* test for paired variates was used with n = number of experiments. Data from different groups were compared using a *t* test for unpaired variates.

RESULTS

Free urine flow studies. Both urine flow and sodium excretion increased abruptly following the administration of ethacrynic acid. The presence of the catheter in the left renal vein had no demonstrable effect on the onset or the magnitude of the natriuresis exhibited by the left kidney. Urine flow rates, sodium excretion, and RVRA before and after the administration of ethacrynic acid in a typical experiment are shown in Table I. Natriuresis was maximal in all experiments during the first 10–20 min after ethacrynic acid was administered, reaching a mean level of 611 μEq/min, SEM ±140, on the left side and 568.3 μEq/min, SEM ±66, on the right. After this maximal response, sodium excretion gradually diminished. The mean rate of sodium excretion during the final collection period, however, was substantially greater than the prediuresis control values.

RVRA increased within 5 min after the administration of ethacrynic acid and remained elevated. The highest values were achieved during the period of maximal natriuresis, and lower values were obtained from samples of renal venous blood collected as natriuresis was subsiding.

The values of RVRA (nanograms angiotensin II formed per 100 ml plasma) in this group of experiments, before and after the administration of ethacrynic acid, are shown in Table II. RVRA, following the administration of ethacrynic acid, increased within 5 min from a mean control value of 621, SEM ±54 to 1613, SEM ±321 ($P < 0.05$). The mean RVRA in samples of renal venous blood obtained during the first 15 min after the drug was administered was 1787, SEM ±286. The difference between RVRA in these samples and RVRA in the control samples was also significant ($P < 0.02$).

Reinfusion studies. External sodium loss and volume depletion were prevented in this group of experiments by insertion of the ureteral catheters into the femoral veins immediately after the initiation of drug-induced diuresis. RVRA increased, as in the previous group of experiments, within 5 min after the drug was administered and remained elevated despite the lack of change in fluid volume and sodium balance.

Individual and mean values for RVRA, before and after the administration of ethacrynic acid, are shown in Table III. Within 5 min after the administration of

TABLE II

*Renin Activity in Renal Venous Blood (ngm Angiotensin II Formed per 100 ml Plasma)
before and after the Administration of Ethacrynic Acid in Free Urine Flow Studies*

Exp. No.	Before ethacrynic acid			After ethacrynic acid				
	15 min	10 min	5 min	5 min	10 min	15 min	30 min	60 min
1	650	1025	437	2300	2600	2550	2500	3000
2	500	500	600	750	1150	1350	1150	700
3	850	800	515	1750	1900	1700	2580	1540
4	475	650	450	1650	1750	2000	2200	1300
Mean* ±SEM	621 ±54			1787 ±286				

* Means (±SEM) are means of the average values for RVRA in the control period (before ethacrynic acid) and during the first 15 min after the drug was administered (n = 4).

ethacrynic acid, RVRA increased from a mean control value of 1797, SEM ±291 to 4081, SEM ±532 (P < 0.02). The mean RVRA (4431, SEM ±475) in the samples of blood obtained in the first 15 min after the administration of ethacrynic acid was also significantly greater (P < 0.005) than the mean RVRA in the samples obtained in the preceding control period.

When chlorothiazide was administered in comparable studies instead of ethacrynic acid, RVRA increased

transiently in two of the experiments and was unaltered in four (Table III). Neither the mean level of RVRA (2320, SEM ±768) in the samples obtained within the first 5 min after chlorothiazide was administered (P > 0.2) nor the mean RVRA (1893, SEM ±620) in the samples obtained during the first 15 min after the drug was administered (P > 0.3) was significantly different from the mean control level of 1458, SEM ±360. As shown in Fig. 1, the effect of chlorothiazide on RVRA

TABLE III

*Renin Activity in Renal Venous Blood (ngm Angiotensin II Formed per 100 ml Plasma)
before and after the Administration of Ethacrynic Acid or Chlorothiazide
in Reinfusion Studies*

Exp. No.	15 min	10 min	5 min	5 min	10 min	15 min	30 min	60 min
	Before ethacrynic acid			After ethacrynic acid				
1	2125	1400	1600	5200	8000	6600	10000	4100
2	2000	2000	2000	2050	3600	3600	5700	3500
3	3750	3200	2750	3200	4500	4800	4400	3000
4	1650	2300	1750	3600	3600	3400	3000	2000
5	2500	2700	2600	4200		6000	9800	5900
6	1000	1100	1000	2900	3700	2000	3700	3300
7	940	940	1175	6900	6200	4800	4500	6600
8	950	800	900	4600	4350	3450	5850	5450
Mean* ±SEM	1797 ±291			4431 ±475				
	Before chlorothiazide			After chlorothiazide				
1	2180	2020	3030	3570	2350	3280	3530	2620
2	1400	2500	2100	5400	3880	3700	2200	1850
3	350	400	350	450	450	400	500	425
4	850	650	600	650	450	450	750	650
5	2700	1800	2400	1600	1250	1400	1300	1000
6	1035	915	960	2250	1375	1165	1375	1525
Mean* ±SEM	1458 ±360			1893 ±620				

* Means (±SEM) are means of the average values for RVRA in the control period (before ethacrynic acid or chlorothiazide) and during the first 15 min after the drugs were administered (n = 8 for ethacrynic acid studies and 6 for chlorothiazide studies).
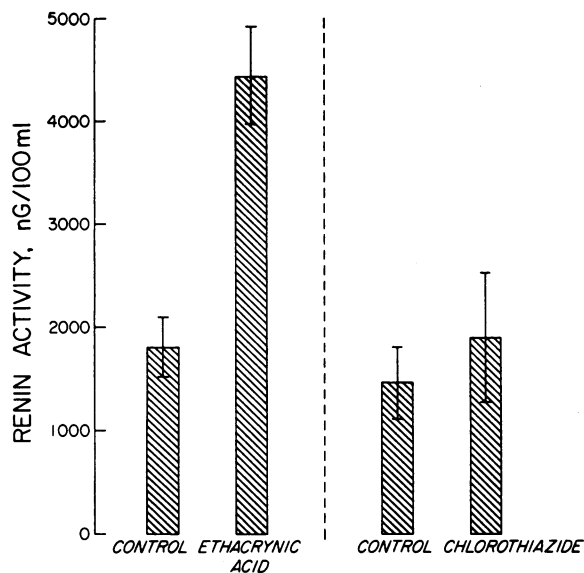
FIGURE 1 Reinfusion studies; comparison of the effects of ethacrynic acid and chlorothiazide on RVRA during the first 15 min after drug administration. Bars represent mean values ±1 SEM.

during the first 15 min after the drug was administered was substantially different from the effect of ethacrynic acid. This difference was significant at the 2% level.

Ureteral occlusion studies with ethacrynic acid. In this group of experiments, ethacrynic acid was administered during a period in which tubular flow was temporarily interrupted by ureteral occlusion.

The results of these studies are shown in Table IV. The mean control value for RVRA before ureteral occlusion was 1829, SEM ±227 and the mean RVRA in samples of renal venous blood collected during the period of occlusion before the administration of ethacrynic acid was 4316, SEM ±1031 ($P > 0.05$). Ethacrynic acid administration, with the ureters occluded, produced no

further increase in RVRA. Following release of the occlusion and restoration of urine flow, however, mean RVRA increased from 4754, SEM ±875 to 7488, SEM ±1306 ($P < 0.05$).

Urine excreted following release of the occlusion was collected serially in 1.0 ml aliquots. The concentration of sodium in these samples (Fig. 2) was measured as a rough index of the concentration of sodium in distal nephron segments during the period of ureteral occlusion and following the restoration of urine flow. The concentration of sodium in the first 5–8 ml of urine collected, which was the urine "trapped" in distal nephron segments during the period of occlusion, was substantially lower than the concentration of sodium in subsequent samples, which represented proximal stop-flow urine and new filtrate formed following the resumption of urine flow. The low sodium urine samples were excreted within the first 1–2 min after ureteral release, and the first postrelease samples of renal venous blood were obtained 5 min after ureteral release, by which time the concentration of sodium in the urine had increased to maximal levels.

Ureteral occlusion studies without ethacrynic acid. As a control for the preceding group of experiments, the effects of ureteral occlusion and restoration of urine flow on renin release were also studied without the administration of ethacrynic acid. The results of these studies are shown in Table V. The RVRA in the samples obtained while the ureters were occluded was higher than the level of RVRA in the preceding control period in three of these experiments and unchanged in the fourth. The mean RVRA during ureteral occlusion was 2748, SEM ±808. This increase, however, did not prove to be significant ($P > 0.1$). In contrast with the increase in RVRA that occurred when urine flow was restored in the studies performed with ethacrynic acid, there was no further increase in RVRA in the postrelease period in this group of experiments (Fig. 3). Instead,

TABLE IV

Renin Activity in Renal Venous Blood (ngm Angiotensin II Formed per 100 ml Plasma) before and after the Administration of Ethacrynic Acid during Ureteral Occlusion

Exp. No.	Before occlusion			Ureters occluded						Postrelease		
				Before EA			After EA					
1	2150	2500	2250	6600	7000	7000	7000	6000	8000	12500	9900	9900
2	2200	2000	2200	2600	2400	4050	2200	2700	3750	4600	5450	6400
3		1400	1500	2200	2550	2200	4100	4000	4000	7400	8000	9240
4	950	1600	1750	7600	3500	4100	4100	5600	5600	3250		7800
Mean* ±SEM	1829 ±227			4316 ±1031			4754 ±875			$P < 0.05$ 7488 ±1306		

EA = ethacrynic acid.
* Means (±SEM) are means of the average values for RVRA in each period of the study (n = 4).

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RVRA, in the postrelease period, was consistently lower than the RVRA in the preceding period of ureteral occlusion.

The data obtained in these two groups of experiments involving ureteral occlusion are shown for comparison in Table VI. When ethacrynic acid was administered during the period of ureteral occlusion the mean RVRA in the samples obtained in the postrelease period was 7488, SEM ±1306, whereas the mean RVRA after flow was restored following ureteral occlusion without the administration of ethacrynic acid was 1153, SEM ±219. This difference is significant at the 1% level. As compared with the values for RVRA during ureteral occlusion, there was a mean increase in RVRA in the postrelease period of 2739, SEM ±839 when ethacrynic acid was administered during the occlusion, and a mean decrease of 1595, SEM ±664 when no drug was administered. This difference between these two groups is likewise significant ($P < 0.01$).

DISCUSSION

Despite considerable variation in the RVRA at the beginning of the studies, consistent data were obtained in each group of experiments. The wide range of control values is probably attributable to a variety of factors. However, it is likely that the most significant factor, as suggested by the studies of Bunag, Page, and McCubbin (4), is a lack of uniformity in the animals' prior dietary sodium intake. The renin responses to ethacrynic acid administration were of the same order of magnitude regardless of the basis for the differences observed in the initial control values. In the free urine flow studies and the reinfusion studies with ethacrynic acid, the difference between control values and the RVRA in the 5-min samples was 1537, SEM ±872 in the five individual experiments in which the mean control value for RVRA was less than 1000 ngm angiotensin II formed per 100 ml plasma and 2080, SEM ±773 in the seven experiments in which the mean control value was higher than this.
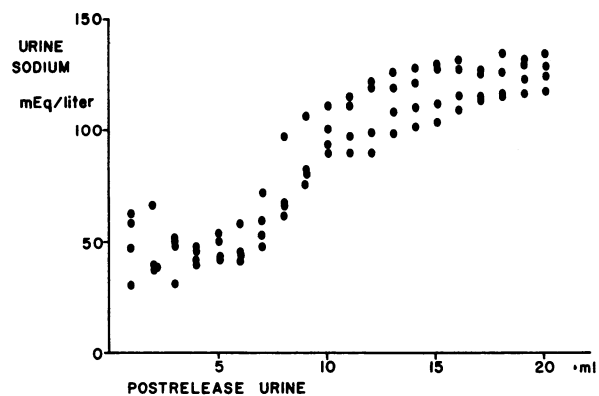

FIGURE 2 Sodium concentration in 1.0 ml aliquots of "stop flow" urine collected after release of the occlusion in the ureteral occlusion studies with ethacrynic acid.

Following the administration of ethacrynic acid, and coincident with the onset of natriuresis in the free urine flow studies, the renin activity in the renal venous blood increased abruptly. This effect was observed within the first 5 min after the drug was administered, during which time the average volume of urine excreted was only 40 ml and the average quantity of sodium excreted was 5 mEq. Thus, it seems entirely unlikely that the initial marked increase in RVRA in these free urine flow studies was related to changes in volume. The effect of volume depletion on RVRA was further excluded in the group of experiments in which the urine was shunted into the femoral veins immediately following the onset of natriuresis. RVRA, in these reinfusion studies, increased promptly and remained significantly higher than the predrug control levels in spite of the fact that volume depletion was prevented.

These findings suggest that ethacrynic acid stimulates renin release either by acting directly upon the juxtaglomerular apparatus or by effecting a change in tubular fluid sodium. It is conceivable that ethacrynic acid could produce this effect by altering some aspect

TABLE V

Renin Activity in Renal Venous Blood (ngm Angiotensin II Formed per 100 ml Plasma) before, during, and after Ureteral Occlusion without the Administration of Ethacrynic Acid

Exp. No.	Before occlusion			Ureters occluded			Postrelease		
1	2600	1600	1800	4000	5600	5600	1800	1400	1300
2	800	900	1000	2200	2000	2800	900	1500	900
3	2600	2450	2175	2300	2250	2300	1560	1475	1350
4	750	510	450	1280	1600	1050	640	570	450
Mean* ±SEM	1465 ±435			2748 ±808			1153 ±219		

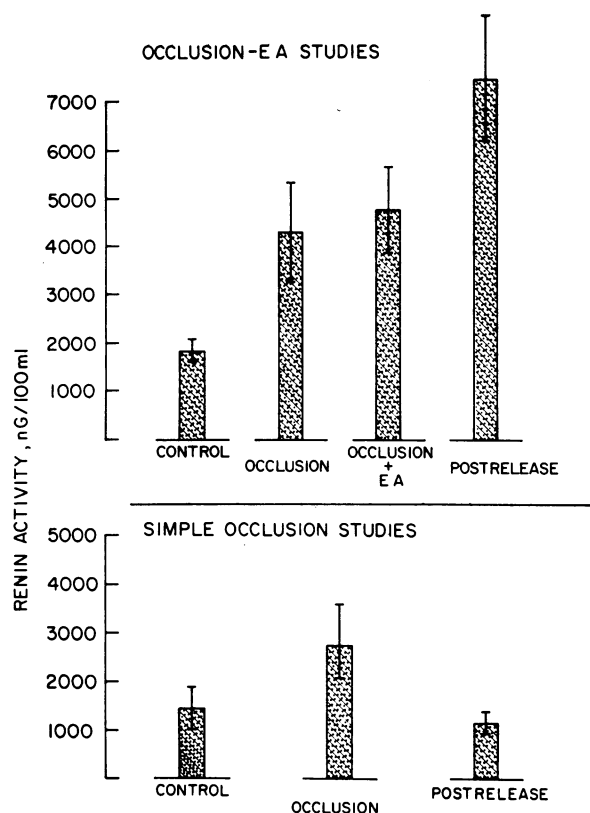* Means (±SEM) are means of the average values for RVRA in each period of the study (n⁻ = 4).

FIGURE 3 Comparison of the data from the ureteral occlusion studies performed with and without ethacrynic acid. Bars represent mean values ±1 SEM.

of the renal circulation, i.e., in a manner that would be consistent with the baroreceptor hypothesis proposed by Tobian (24). Redistribution of renal blood flow has been demonstrated following the administration of ethacrynic acid by Birtch, Zakheim, Jones, and Barger (25). However, in our ureteral occlusion studies with ethacrynic acid, no response was observed while the ureters were occluded. Since total renal blood flow remains normal or is increased during ureteral occlusion (26), the drug should have had free access to the renal vasculature, and if the stimulus provided by ethacrynic acid were a change in renal blood flow, the effect of ethacrynic acid on RVRA should not have been delayed until the occlusion was released. Thus, the alternate possibility that ethacrynic acid produces an increase in renin release by increasing the sodium concentration in the distal convolutions merits further consideration.

When chlorothiazide was administered instead of ethacrynic acid, and the urine was shunted into the femoral veins, RVRA was essentially unaltered. RVRA was transiently increased in two of the experiments, but the mean RVRA in the samples obtained within the first 15 min after chlorothiazide was administered was not

significantly different from the mean control level. This failure of chlorothiazide to stimulate an increase in RVRA in the absence of drug-induced volume depletion is consistent with the findings of Brown, Davis, and Johnston (27), who observed that although chlorothiazide and Mercuhydrin (meralluride) both caused an increase in plasma renin activity when urinary losses were not promptly replaced, neither chlorothiazide nor Mercuhydrin consistently increased plasma renin activity when sodium and water losses were simultaneously replenished. Unlike ethacrynic acid, which produces prompt inhibition of tubular reabsorption of solute-free water ($T^{c}_{H_2O}$) during antidiuresis as well as inhibition of free water clearance (C_{H_2O}) during water diuresis (17, 18), chlorothiazide has been shown to inhibit C_{H_2O} without also diminishing $T^{c}_{H_2O}$ (20, 21). These observations have suggested that the locus of action of ethacrynic acid includes the medullary portion of the ascending limb of the loop of Henle, whereas chlorothiazide acts mainly in a more distal diluting segment which does not play a role in the renal concentrating mechanism. Although Gottschalk has shown that dilution of the urine is virtually complete before the tubular fluid enters the distal convolutions (28), which suggests that chlorothiazide acts, as does ethacrynic acid, proximal to the site of the macula densa, the greater reduction of C_{H_2O} produced by ethacrynic acid (29) and its additional inhibitory effect upon $T^{c}_{H_2O}$ both suggest that more sodium is delivered in higher concentrations to the distal convolutions by ethacrynic acid than by chlorothiazide. Furthermore, Clapp and Robinson observed, in micropuncture studies (30), that the mean distal osmolal TF/P ratio after furosemide administration, which produces effects that are comparable to those of ethacrynic acid (31), was 0.83, whereas the mean distal osmolal TF/P ratio after chlorothiazide administration was 0.48. It is also of interest, in this regard, that Meyer, Menard, Papanicolaou, Alexandre, Devaux, and Milliez observed an increase in plasma renin activity after

TABLE VI

Comparison of Data from Ureteral Occlusion Studies with Ethacrynic Acid (I) with the Data Obtained from Ureteral Occlusion Studies without Ethacrynic Acid (II)

	I	II	I vs. II
Before occlusion	1829 ±227	1465 ±435	$P > 0.5$, NS
Ureters occluded	4316 ±1031	2748 ±808	$P > 0.3$, NS
Occlusion + EA	4754 ±875		
Postrelease	7488 ±1306	1153 ±219	$P < 0.01$
Difference (postrelease-occlusion)	+2739 ±839	−1595 ±664	$P < 0.01$

EA = ethacrynic acid.
Values are mean RVRA (ngm angiotensin II formed per 100 ml plasma) ±SEM.

furosemide administration in their studies on rabbits in which volume changes were excluded as in our reinfusion studies with ethacrynic acid (10).

The possibility that ethacrynic acid produces a stimulus for renin release as a result of its action on tubular sodium transport is further suggested by our findings in the studies performed with ureteral occlusion. As shown in Fig. 3, when ethacrynic acid was administered while the ureters were occluded, there was no further increase in RVRA beyond that produced by the occlusion alone until flow was restored by releasing the occlusion. Although, as previously shown by Vander and Miller (9), ureteral occlusion also stimulates renin release, the effect of the occlusion on RVRA does not persist after flow is restored. In our ureteral occlusion studies without ethacrynic acid, RVRA returned promptly to the previous control levels when the occlusion was released in marked contrast with the increase in RVRA in the postrelease period when ethacrynic acid was administered during the period of occlusion. Although some distal movement of tubular fluid continues after the ureters are occluded, probably on the basis of continued reabsorption of solute and water and new filtrate formation (32), tubular flow virtually ceases after 10 or 12 min as poorly reabsorbed solute accumulates within the tubular lumen (33). In the present experiments, ethacrynic acid was administered 15 min after ureteral occlusion and another 15 min was allowed to elapse before the occlusion was released. Furthermore, the inhibitory effect of ethacrynic acid on sodium reabsorption should have decreased the rate of new filtrate formation during the period of occlusion by reducing the loss of tubular volume. Indirect evidence of stopped tubular flow in the period of ureteral occlusion that followed the administration of ethacrynic acid in these studies is provided by the finding of low sodium concentrations in the early stop-flow samples as shown in Fig. 2. The low sodium concentration in the early stop-flow samples should, in part, reflect the continuance of sodium reabsorption in the ascending limb of the loop of Henle during the first 15 min of the period of occlusion, which was prior to the administration of ethacrynic acid. Continued distal movement of tubular fluid after the administration of ethacrynic acid during ureteral occlusion would have resulted in the admixture of tubular fluid from the site of drug action with tubular fluid in the distal nephron segments, thereby diminishing the difference between the sodium concentration in the early stop-flow samples and the sodium concentration in the samples derived from more proximal segments. It would thus appear to be a valid assumption that any increment in the concentration of tubular fluid sodium resulting from the action of ethacrynic acid on the ascending

limb of the loop of Henle during ureteral occlusion was retained in this segment until flow was restored.

Studies reported by Gussin and Cafruny have suggested that ethacrynic acid is secreted into the luminal fluid of proximal convolutions and reaches the site of its major effect on sodium reabsorption via the tubular lumen (34). Support for this mechanism of action was provided by the finding that the effect of ethacrynic acid on the distal stop-flow pattern for sodium was partially blocked by probenecid infusions. In accordance with this concept of the mechanism of action of ethacrynic acid, the apparent lack of influence of ethacrynic acid on RVRA during ureteral occlusion, and the subsequently observed increase in RVRA that followed release of the occlusion in our ureteral occlusion studies, could also be related to the failure of the drug to be transported distally in the tubular fluid until flow was restored. However, regardless of the mechanism, the sodium concentration in the tubular fluid in the distal convolutions should have increased abruptly following release of the occlusion. Although localization of the sensing apparatus is by inference only, these data are consistent with a regulatory mechanism whereby renin is released from the granular cells of the afferent arterioles in response to an increase in the sodium concentration in the tubular fluid at the macula densa as previously proposed (7, 8).

The specific effect of ethacrynic acid on sodium reabsorption in the macula densa segment, if any, is not known. It may be argued, however, that ethacrynic acid may increase renin release by inhibiting sodium reabsorption by the macula densa cells in spite of the increase in the sodium concentration in the tubular fluid. As a consequence of this action of ethacrynic acid, the sodium concentration in the macula densa cells could be either decreased or increased, depending upon whether the drug may exert its effect on the luminal or the antiluminal side of the cell. Since retrograde microinjections of mannitol did not cause collapse of the proximal renal tubule, a phenomenon that was postulated to be dependent upon renin release, in the studies reported by Thurau et al. (7, 8), and since neither mannitol nor urea, both of which should decrease the sodium concentration in the distal convolutions (14), increased renin release in the studies reported by Vander and Miller (9), the possibility that renin release is stimulated by a lowered sodium concentration in the macula densa cells seems unlikely. On the other hand, an inhibitory effect of ethacrynic acid on active sodium transport across the antiluminal cell membrane would produce the same effect as increasing the sodium concentration in the luminal fluid, i.e., the sodium concentration in the macula densa cells would be increased. Thus, regardless of whether the effect of ethacrynic acid on RVRA is assumed to be related to a primary effect on sodium reabsorption in

the macula densa segment, or to the increased concentration of tubular fluid sodium resulting from the action of ethacrynic acid on the ascending limb of the loop of Henle, the data presented here strongly support a role for sodium concentration at some point in the distal nephron as representing at least one of the stimuli for the regulation of renin release.

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