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POSSIBLE ROLE FOR PROSTAGLANDINS IN THE CHLORURESIS OF ACUTE VOLUME EXPANSION

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ABSTRACT Prostaglandins have been postulated to participate in the regulation of salt excretion during acute volume expansion. The present papillary and cortical micropuncture studies were designed to examine the effect of prostaglandin synthesis inhibitors on segmental chloride transport during hydropenia (with and without meclofenamate) and 10% volume expansion (with and without both meclofenamate and indomethacin). Both inhibitors significantly decreased the urinary excretion rate of prostaglandins E_2 and $F_{2\alpha}$. Clearance studies on the intact right kidney demonstrated no effect of either agent on glomerular filtration rate, but a significant reduction in chloride excretion during hydropenia and volume expansion was observed. To assess the specific site(s) of enhanced chloride reabsorption, absolute and fractional chloride delivery was measured in the late proximal tubule, thin descending limb of Henle, and the early and late distal tubules. In addition, the fraction of filtered chloride remaining at the base and tip of the papillary collecting duct was compared to that fraction remaining at the superficial late distal tubule. During hydropenia, meclofenamate had no effect on fractional chloride delivery out of the superficial late distal tubule or the juxtamedullary thin descending limb of Henle, but significantly reduced the fraction of chloride delivered to the base of the papillary collecting duct. During volume expansion, neither meclofenamate nor indomethacin had an effect on absolute chloride delivery out of the proximal tubule or the thin descending limb of Henle. However, absolute chloride delivery to the early distal tubule was significantly reduced, and was associated with a decrease in fractional chloride reabsorption in this segment. Furthermore, the fraction of chloride delivered to the base of the collecting duct was significantly reduced. Fractional reabsorption along the terminal 1 mm of the collecting duct was not altered by either meclofenamate or indomethacin. These results suggest that inhibitors of prostaglandin synthesis result in an increase in chloride reabsorption in the superficial loop of Henle, and in segments between the superficial late distal tubule and the base of the collecting duct. The results are consistent with the view that prostaglandins inhibit chloride transport in the thick ascending limb of Henle, and/or the cortical and outer medullary collecting tubule.

INTRODUCTION

It is widely appreciated that acute isotonic volume expansion results in an increase in urinary sodium chloride excretion. However, the mechanism by which this saliuresis is accomplished is not completely understood. Recently, it has been suggested that the terminal nephron segments (distal convoluted and collecting tubules) may play a major role in modulating the magnitude of this saliuresis. Previous studies from our laboratory (1), and from Stein and associates (2) have provided evidence that the juxtamedullary nephrons contribute

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a proportinately greater load of sodium chloride than the superficial nephrons to the final urine during volume expansion.

It has been suggested that nonsteroidal antiinflammatory drugs may promote salt retention (3–5). Furthermore, these agents are generally believed to act by inhibiting endogenous prostaglandin production. Therefore, if prostaglandins play a role in the saliuresis of acute volume expansion, inhibiting their production should blunt the associated saliuresis. The present study was designed to examine this hypothesis by the admission of either meclofenamate or indomethacin to both hydropenic and acutely volume-expanded rats. The site of action of these agents was evaluated by micropuncture of both the accessible portions of the superficial cortex and the surgically exposed papilla of the mutant Munich-Wistar rat. The results demonstrate that inhibition of prostaglandin production results in an increase in chloride reabsorption in the thick ascending limb of Henle, and the cortical and outer medullary collecting tubules. Furthermore, the observed increase in the fraction of chloride delivered out of the juxtamedullary nephrons during volume expansion is blunted after administration of these agents. The results are consistent with the view that endogenous prostaglandins can inhibit chloride reabsorption in these segments and contribute in this manner to the observed increase in chloride delivery out of the juxtamedullary population of nephrons during isotonic volume expansion.

METHODS

Preparation of rats for micropuncture

Studies were performed after 100 mg/kg, i.p. Inactin anesthesia (Promonta, Hamburg, West Germany) on 54 young mutant Munich-Wistar rats weighing 98–160 g. All rats were allowed free access to tap water and standard rat chow (Lab-Blox, Wayne Feeds, Chicago, Ill.) which contained 0.23% Na, 1.1% K, and 0.59% Cl, until the time of the experiment. The rat was placed on a thermostatically controlled heating table and maintained at 37.5°C. After tracheostomy, polyethylene catheters (PE 50) were inserted into the left jugular vein for infusion, into the left femoral artery for constant blood pressure monitoring (strain gauge AMP, Sanborn, Co., Waltham, Mass.) and blood collection, and into the bladder for urine collection. The left kidney was then gently separated from the adrenal gland and peritoneal attachment. The renal papilla was exposed by temporarily displacing the papilla into the renal pelvis and carefully excising the ureter. The kidney was then placed in a Lucite cup surrounded by 3% agar. Previous studies from our laboratory have demonstrated that use of agar in this manner does not result in discrepant renal function between right and left kidneys during hydropenia or volume expansion (6). The kidney was continuously bathed with water-equilibrated mineral oil maintained at 37°C and illuminated with a small fiber optic light source. This technique has been previously described in detail (1). After jugular vein cannulation, rats were infused with Ringer's bicarbonate (Na = 140, Cl = 115, HCO_3 = 30, K = 5 meq/liter) throughout surgery at 1.8 ml/h.

After completion of surgery, [*carboxyl*-^{14}C]insulin (New England Nuclear, Boston, Mass.) was infused at 40–60 μCi/h. During the 1-h inulin equilibration period, the proximal transit time was measured with lissamine green dye (30 μl of 10% solution) and late proximal, early distal, and late distal segments (three to five each) were identified by the technique described by Wright (1) and utilized in our laboratory (6). Eight late distal segments localized by this technique were subsequently microdissected and all of them were found to be within the last 25% of the distal tubule. This finding is compatible with our previous studies (1). Proximal tubular transit times >12 s or a mean arterial blood pressure <90 mm Hg resulted in rejection of the animal at this point.

1 h after initiation of the inulin infusion, and at least 30 min after the lissamine green injection, tubular fluid, urine, and blood sample collections were initiated. Two to three 30-min clearance periods from the untouched right kidney were obtained in each animal.

Micropuncture techniques

The tubule sample collections were performed using sharpened micropipettes the tip diameter of which was 9–11 μm for proximal punctures, 7–9 μm for distal and loop of Henle punctures, and 10–12 μm for collecting duct punctures. The sample collections from proximal and distal tubules were performed after placing Sudan black-colored mineral oil blocks, whereas collecting duct samples were collected by gentle aspiration at a rate slower than tubular fluid flow rate but without an oil block. A limb of Henle was punctured near the base of papilla, and a small drop of oil was injected to ascertain the direction of flow. The order of tubule puncture was randomized to obviate any systematic source of bias.

Protocol

Hydropenic controls (n = 9). The infusion rate was maintained at 1.8 ml/h throughout the experiment. Paired base and tip punctures from the same collecting duct were performed before and after cortical late distal tubule punctures. Two to three late distal samples, one to two samples from the ascending limb of Henle, and two to three paired collecting duct samples were obtained in each animal.

Hydropenia plus meclofenamate (n = 7). Rats which were to receive meclofenamate (Parke, Davis & Co., Detroit, Mich.) were infused with Ringer's bicarbonate at a rate of 1.8 ml/h. Meclofenamate was administered as a loading dose of 3 mg/kg before, and 3 mg/kg after completion of the surgery, both at a rate of 0.03 mg/min. Meclofenamate was then maintained as a sustaining infusion at 4 mg/kg per h.

Volume expansion controls (n = 15). After initial surgery this group of rats received Ringer's bicarbonate to 10% of body weight over a 60-min period. The infusion rate was then decreased to a rate which slightly exceeded the urinary flow rate to maintain the diuresis. Since rats expanded with Ringer's bicarbonate (n = 10) did not differ statistically from rats expanded with the indomethacin carrier (see below), these two groups were pooled as volume expansion controls (n = 15). Micropuncture samples were obtained from superficial late proximal and early and late distal segments, whereas papillary samples were obtained from the thin limb of Henle's loop and the base and tip of the collecting duct.

Volume expansion plus meclofenamate or indomethacin. Meclofenamate (n = 14) was administered to volume-expanded rats in a similar manner as described for hydropenic rats. 50 mg indomethacin (Merck Sharp & Dohme, West Point, Pa.) was dissolved in 0.5 ml of 0.5 M Na_2CO_3; 2 ml of H_2O was then added, followed by 2.0 ml of 0.05 M HCl, and an

appropriate amount of Ringer's bicarbonate to yield a final concentration of 2.7 mg/ml. Indomethacin ($n = 9$) was infused 30 min before (5 mg/kg) and just after (3 mg/kg) opening the renal pelvis at a rate of 0.047 mg/min. A sustaining infusion of indomethacin (6 mg/kg per h) was begun simultaneously with Ringer loading.

Analytical techniques

Inulin concentration in tubular fluid, urine, and plasma was measured by counting ^{14}C activity in a liquid scintillation counter (Packard Tri-Carb, Packard Instrument Co., Inc., Downers Grove, Ill.). Chloride concentration in tubular fluid was determined by the microcoulometric method of Ramsay et al. (8). Chloride concentrations in plasma and urine were measured by a Buchler-Cotlove chloridometer (Buchler Instruments, Div., Searle Diagnostics Inc., Fort Lee, N. J.).

Radioimmunoassay of urinary prostaglandins E_2 and $F_{2\alpha}$

The concentration of prostaglandins (PG)[1] E_2 and $F_{2\alpha}$ in urine samples obtained from the same volume-expanded animals used for micropuncture were measured according to the method of Dray et al. (9). This technique requires acid-lipid extraction, silicic acid chromatography, and radioimmunoassay. The antibody for PGE_2 cross-reacted 50% with PGE_1 and <0.5% with $PGF_{2\alpha}$, PGA_2, PGB_2, and its 15-keto metabolites. A similar antibody specificity was observed with antiserum against $PGF_{2\alpha}$.

Determination of papillary tissue chloride concentration

After micropuncture, the renal papilla was quickly removed and placed in a preweighed bottle. After determination of the wet weight, the tissue was dried at 60°C for 48 h and then reweighed for determination of water content. The tissue was then extracted with water at 80°C for 2 h. The chloride concentration of this fluid was then measured with a Buchler-Cotlove chloridometer.

Calculations

(*a*) Fraction (percent) of filtered chloride remaining at a given segment:

$$(TF/P)_{Cl/In} \times 100.$$

(*b*) Fraction (percent) of filtered chloride reabsorbed up to the late proximal or ascending limb of Henle:

$$1 - [(TF/P)_{Cl/In}] \times 100.$$

(*c*) Fraction (percent) of filtered chloride reabsorbed between two nephron segments A and B:

$$[(TF/P)_{Cl/In}{}^{A} - (TF/P)_{Cl/In}{}^{B}] \times 100.$$

This calculation was performed using mean values. Values for the collecting duct were corrected by the distance (millimeters) between the base and tip collecting sites.

(*d*) Absolute chloride reabsorption was calculated from the distal single nephron glomerular filtration rate, nephron filtered load, and the appropriate values for fractional reabsorption as calculated above.

RESULTS

Whole kidney data. Table I displays the data for whole kidney clearance, hematocrit, and plasma chloride concentrations in each group. Whole kidney clearance results were obtained from the untouched right kidney. Urine volume was decreased significantly by both meclofenamate and indomethacin during both hydropenia and volume expansion. The glomerular filtration rate, however, was not affected by either meclofenamate or indomethacin. Urinary chloride excretion decreased ≅50% after either meclofenamate or indomethacin during hydropenia (5.4 vs. 2.0 μeq/min per kg body wt, $P < 0.001$) and volume expansion (35.3 in control, 18.2 with meclofenamate, and 16.6 μeq/min per kg body wt with indomethacin). Similar decreases in urinary fractional chloride excretion were obtained. No significant change in hematocrit or plasma chloride concentration was observed in groups treated with indomethacin or meclofenamate.

Micropuncture data. Tables II–IV and Figs. 1–4 display the data obtained by micropuncture of the left experimental kidney. Single nephron glomerular filtration rates obtained from distal tubular punctures were not altered by meclofenamate or indomethacin (Table II). Furthermore, because we have previously demonstrated (10) that inhibition of prostaglandin production does not significantly alter juxtamedullary single nephron filtration rate in either hydropenia (52.8±4.2 vs. 47.8±5.8) or volume expansion (48.4±1.9 vs. 53.2±2.6), all subsequent juxtamedullary data are expressed in fractional terms only.

Proximal tubule. The fraction of chloride remaining at the superficial late proximal tubule was not altered by either meclofenamate or indomethacin during acute volume expansion (Table II). Therefore, fractional chloride reabsorption in the superficial proximal tubule was not altered by prostaglandin inhibition.

Thin limb of Henle. The fraction of filtered chloride remaining at the thin limb of juxtamedullary nephrons was not affected by meclofenamate during hydropenia (Table II) although the chloride concentration ratio increased from 2.47 to 4.10 ($P < 0.001$, Table III). Similarly, the fraction of chloride remaining at this site during volume expansion was not changed after meclofenamate or indomethacin. Thus, meclofenamate and indomethacin did not alter chloride reabsorption up to the thin limb of the juxtamedullary nephron in either hydropenia or volume expansion.

Superficial loop of Henle. The fraction of chloride delivered to the early distal tubule was significantly decreased by both meclofenamate (18.5 vs. 12.7%, $P < 0.001$) and indomethacin (14.4%, $P < 0.02$) during

[1] *Abbreviations used in this paper*: PGE_2, $PGF_{2\alpha}$, prostaglandins E_2, $F_{2\alpha}$.

TABLE I
Clearance Results of the Untouched Right Kidney

		Urine volume	$(U/P)_{In}$	GFR	$(U/P)_{Cl}$	$U_{Cl}V$	FE_{Cl}	Hct	P_{Cl}
		μl/min/kg body wt		*ml/min/kg body wt*		*μeq/min/kg body wt*	*%*	*%*	*meq/liter*
Hydropenia Control (9)	Mean	17.1	374.1	6.04	3.07	5.4	0.89	48.3	103.7
	±SE	1.4	30.3	0.34	0.18	0.5	0.09	0.8	2.4
Hydropenia + meclofenamate (7)	Mean	10.8	595.7	6.02	2.48	2.9	0.48	46.6	105.9
	±SE	1.4	64.4	0.61	0.24	0.5	0.09	0.3	1.7
	P	<0.02	<0.01	NS	NS	<0.01	<0.02	NS	NS
Volume expansion Control	Mean	205.6	42.8	6.45	1.94	35.3	5.41	43.5	103.4
	±SE	27.7	4.6	0.26	0.13	3.3	0.54	0.7	1.6
Volume expansion + meclofenamate (15)	Mean	104.1	93.5	6.61	1.66	18.2	2.51	43.0	107.6
	±SE	14.5	26.7	0.18	0.17	2.7	0.36	0.3	1.2
	P	<0.01	<0.05	NS	NS	<0.002	<0.001	NS	NS
Volume expansion + indomethacin (9)	Mean	81.5	87.5	5.80	2.12	16.6	3.09	41.8	108.5
	±SE	13.1	18.1	0.32	0.12	2.5	0.41	1.2	1.6
	P	<0.005	<0.002	NS	NS	<0.001	<0.01	NS	NS
	*P**	NS	NS	<0.05	NS	NS	NS	NS	NS

Abbreviations used in this table: $(U/P)_{In}$, urine to plasma inulin concentration ratio; GFR, glomerular filtration rate; $(U/P)_{Cl}$, urine to plasma chloride concentration ratio; $U_{Cl}V$, urinary chloride excretion; FE_{Cl}, fractional excretion of chloride; Hct, hematocrit; P_{Cl}, plasma chloride concentration.
Numbers in parentheses are the number of animals.
* *P* value compares meclofenamate to indomethacin during volume expansion.

volume expansion (Table II, Fig. 1). Therefore, the fraction of chloride reabsorbed between the late proximal tubule and the early distal tubule (short loop of Henle) increased from 53.1±1.7 to 57.3±1.4% (NS) after meclofenamate and to 60.2±1.6% ($P < 0.02$) after indomethacin (Table IV, Fig. 2). Although the increase after meclofenamate was not statistically significant, the findings with meclofenamate and indomethacin did not differ from each other and thus the direction of change with both inhibitors was similar. Similarly, the absolute amount of chloride reabsorbed in this segment increased from 2.02±0.18 (volume expansion) to 2.70±0.18 peq/min during volume expansion plus meclofenamate or indomethacin ($P < 0.05$). The chloride concentration ratio at the early distal tubule was significantly less after meclofenamate (0.4 vs. 0.35, $P < 0.002$) and indomethacin (0.46 vs. 0.39, $P < 0.05$) during volume expansion (Table III).

Distal tubule. The fraction of chloride remaining at the late distal tubule was not changed significantly by melcofenamate or indomethacin during hydropenia or volume expansion (Table II, Fig. 1). The fraction of chloride reabsorbed between the early and late distal tubule decreased, however, from 11.0 to 7.0% after meclofenamate and to 5.5% after indomethacin during volume expansion (Table IV). If fractional reabsorption in the distal tubule is expressed in terms of the fraction of the delivered load reabsorbed, 56.8±1.0% was reabsorbed during volume expansion, 46.5±1.0% during volume expansion plus meclofenamate ($P < 0.05$), and 42.4±1.4% during volume expansion plus indomethacin ($P < 0.02$). It should also be noted that a steeper chloride concentration ratio was generated at the late distal tubule after both meclofenamate and indomethacin during volume expansion (Table III).

Superficial late distal tubule to base collecting duct. The results comparing the fraction of filtered chloride remaining at the late distal tubule to that fraction remaining at the base of the collecting duct are displayed in Figs. 3 and 4. As demonstrated in Fig. 3, there was no significant difference in the fraction of chloride remaining at either of these two nephron sites during hydropenia or after administration of meclofenamate. However, the fractional reabsorption observed after meclofenamate is significantly different than the small fractional addition observed during the control period ($P < 0.05$). In addition, the fraction of chloride remaining at the base of the collecting tubule

TABLE II

Micropuncture Results. Single Nephron Glomerular Filtration Rate and Fractional Chloride Delivery to Each Nephron Segment

			Fractional chloride delivery $(TF/P)_{Cl/In}$, %						
		SNGFR*	ALH‡	LP§	ED§	LD§	BCD‡		TCD‡
		nl/min							
Hydropenia	Mean	26.4	44.8			2.2	2.9		1.7
Control	±SE	0.8	3.8			0.4	0.4	$P^{\parallel} < 0.001$	0.1
	(*n*)	(12)	(14)			(12)	(9)		
Hydropenia	Mean	27.7	46.6			2.8	1.6		1.0
+ meclofenamate	±SE	1.0	4.0			0.5	0.2	$P^{\parallel} < 0.001$	0.1
	(*n*)	(13)	(7)			(13)	(17)		(17)
	P	NS	NS			NS	<0.005		<0.005
Volume expansion	Mean	30.9	62.1	72.4	18.5	8.0	15.0		12.3
Control	±SE	1.0	2.4	1.6	1.0	0.8	0.7	$P^{\parallel} < 0.001$	0.8
	(*n*)	(50)	(22)	(22)	(25)	(27)	(37)		(37)
Volume expansion	Mean	32.5	61.4	68.9	12.7	6.8	7.2		4.8
+ meclofenamate	±SE	1.1	3.0	1.8	0.9	0.6	0.5	$P^{\parallel} < 0.001$	0.5
	(*n*)	(42)	(19)	(19)	(19)	(28)	(34)		(34)
	P	NS	NS	NS	<0.001	NS	<0.001		<0.001
Volume expansion	Mean	34.0	68.2	73.7	14.4	8.3	6.1		4.8
+ indomethacin	±SE	2.0	6.0	2.3	1.2	0.7	0.7	$P^{\parallel} < 0.005$	0.7
	(*n*)	(28)	(9)	(16)	(18)	(15)	(10)		(10)
	P	NS	NS	NS	<0.02	NS	<0.001		<0.001
	P¶	NS	NS	NS	NS	NS	NS		NS

SNGFR, single nephron glomerular filtration rate; ALH, thin ascending limb of Henle; LP, late proximal tubule; ED, early distal tubule; LD, late distal tubule; BCD, base of the papillary collecting duct; TCD, tip of the papillary collecting duct. (*n*) refers to number of tubules punctured.

* Obtained by the DT punctures.

‡ Obtained by punctures on the exposed papilla.

§ Obtained by punctures on the cortical surface.

$^{\parallel}$ *P* value compares BCD to TCD by paired *t* test.

¶ *P* value compares meclofenamate to indomethacin during volume expansion.

is less after meclofenamate (1.6%) than during the hydropenic control period (2.9%) ($P < 0.005$).

As is shown in Fig. 4, it is clear that during volume expansion net addition of chloride was observed between the superficial late distal tubule and the base of the collecting duct. The addition of chloride between these two nephron sites during volume expansion was blunted by both meclofenamate and indomethacin. The fraction of chloride remaining at the base of the collecting duct was significantly reduced by meclofenamate (15.0 vs. 7.2%, $P < 0.001$) and indomethacin (15.0 vs. 6.1%, $P < 0.001$). Thus, meclofenamate and indomethacin reversed the fractional addition of chloride observed between the late distal tubule and the base of the collecting duct during volume expansion (Figs. 1, 4).

Papillary collecting duct. The fraction of chloride remaining at the tip of the collecting duct decreased from 1.7 to 1.0% after meclofenamate during hydropenia (Table II). A similar decrease in the fraction of chloride remaining at this site was observed during volume expansion (Table III). Chloride reabsorption between the base and tip of the collecting duct was observed in every experimental group. The fraction of filtered chloride reabsorbed along 1 mm of papillary collecting duct was 1.37% during hydropenia whereas 0.52% was reabsorbed during hydropenia after meclofenamate. The filtered chloride reabsorbed along 1 mm collecting duct during volume expansion, volume expansion with meclofenamate, and indomethacin was 2.4, 1.9, and 1.0%, respectively. Because the magnitude of chloride delivered to the base collecting duct was less with meclofenamate and indomethacin, and because the chloride reabsorption across the papillary collecting duct has been demonstrated to be load dependent (1), the percentage of delivered chloride

TABLE III

Micropuncture Results. Tubular Fluid to Plasma Chloride Concentration Ratio ($TF/P)_{Cl}$

		ALH	LP	ED	LD	BCD	TCD
Hydropenia	Mean	2.47			0.45	1.51	1.56
Control	±SE	0.45			0.05	0.10	0.15
	(*n*)	(14)			(12)	(9)	(9)
Hydropenia	Mean	4.10			0.34	2.34	2.19
+ meclofenamate	±SE	0.45			0.05	0.25	0.25
	(*n*)	(7)			(13)	(17)	(17)
	P	<0.001			NS	<0.05	NS
Volume expansion	Mean	2.07	1.23	0.46	0.41	1.56	1.62
Control	±SE	0.09	0.02	0.02	0.03	0.07	0.08
	(*n*)	(22)	(22)	(25)	(27)	(37)	(37)
Volume expansion	Mean	2.15	1.15	0.35	0.26	1.23	1.20
+ meclofenamate	±SE	0.07	0.01	0.02	0.02	0.08	0.09
	(*n*)	(19)	(19)	(19)	(28)	(34)	(34)
	P	NS	<0.001	<0.002	<0.001	<0.005	<0.005
Volume expansion	Mean	2.84	1.20	0.39	0.28	2.14	2.07
+ indomethacin	±SE	0.23	0.02	0.03	0.02	0.16	0.17
	(*n*)	(9)	(16)	(18)	(15)	(10)	(10)
	P	<0.002	NS	<0.05	<0.005	<0.02	<0.02
	*P**	<0.002	<0.05	NS	NS	<0.001	<0.001

Abbreviations same as Table II.

* *P* value compares meclofenamate to indomethacin during volume expansion.

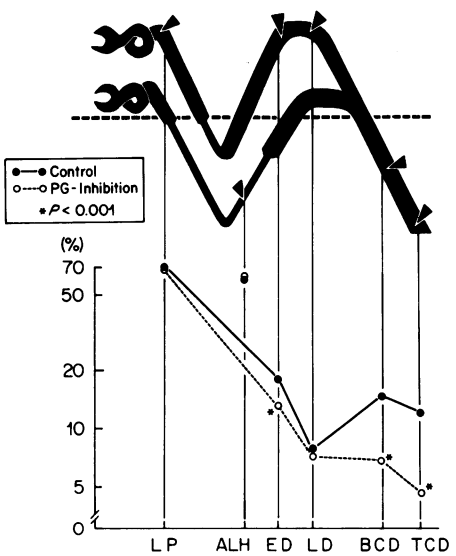

FIGURE 1 The fraction (percent) of filtered chloride remaining at each examined nephron segment during volume expansion. "Control" represents volume expansion controls and "PG-Inhibition" represents the combined results of the volume expansion with meclofenamate and volume expansion with indomethacin groups. The results are shown as mean values. LP, late proximal tubule; ALH, thin ascending limb of Henle; ED, early distal tubule; LD, late distal tubule; BCD, base of the collecting duct; and TCD, tip of the collecting duct.

reabsorbed along the papillary collecting duct was calculated. During hydropenia 41.2±6.6 and 31.5±5.6% (NS) of the chloride delivered to the base collecting duct was reabsorbed along 1 mm in controls and after meclofenamate, respectively. The delivered chloride reabsorbed along 1 mm collecting duct was 16.2±3.4, 24.4±3.4 (NS), and 20.2±4.5% (NS) during volume expansion, volume expansion with meclofenamate, and volume expansion with indomethacin, respectively. Thus, the fraction of chloride remaining at the tip of the collecting duct was reduced with meclofenamate and indomethacin during both hydropenia and volume expansion. The data indicate that both meclofenamate and indomethacin had no effect on chloride reabsorption in the papillary collecting duct, however.

Urinary prostaglandins. The excretion of urinary PGE_2 and $PGF_{2\alpha}$ are displayed in Table V. Urinary excretion of PGE_2 and $PGF_{2\alpha}$ was suppressed ≅95% with both drugs during volume expansion. There was no significant difference in the urinary prostaglandin excretion observed between the meclofenamate- and indomethacin-treated groups.

Papillary tissue chloride concentration. The papillary tissue chloride concentration of the left exposed papilla measured at the end of the micropuncture experiments are shown in Table VI for the respective groups. During hydropenia, meclofenamate administration resulted in a significant increase in chloride

TABLE IV

Micropuncture Results. Fraction of Filtered Chloride Reabsorbed Along Various Nephron Segments during Volume Expansion

		ALH	Proximal convoluted tubule	Short loop of Henle*	Distal convoluted tubule‡	Papillary collecting duct§
				%		%/mm
Volume expansion	Mean	37.9	27.6	53.1	11.0	2.4
Control	±SE	2.4	1.6	1.7	1.0	0.5
	(*n*)	(22)	(22)	(8)	(8)	(37)
Volume expansion	Mean	38.6	31.1	57.3	7.0	1.9
+ meclofenamate	±SE	3.0	1.8	1.4	1.0	0.3
	(*n*)	(19)	(19)	(7)	(7)	(34)
	P	NS	NS	NS	<0.05	—
Volume expansion	Mean	31.8	26.3	60.2	5.5	1.0
+ indomethacin	±SE	6.0	2.3	1.6	1.4	0.3
	(*n*)	(9)	(16)	(8)	(7)	(10)
	P	NS	NS	<0.02	<0.02	—
	P‖	NS	NS	NS	NS	—

Abbreviations same as Table II.

* Mean difference between LP and ED in each kidney.

‡ Mean difference between ED and LD in each kidney.

§ Difference between paired CD and TCD corrected by distance.

‖ *P* value compares meclofenamate to indomethacin group.

concentration (119–215 meq/kg tissue H_2O). Similarly, during volume expansion, both meclofenamate and indomethacin administration were associated with a significant increase in papillary tissue chloride concentration (127±15–181±9 and 220±13 meq/kg tissue H_2O, respectively) ($P < 0.01$ and $P < 0.001$). The increase after indomethacin was more marked than the increase after meclofenamate ($P < 0.05$).

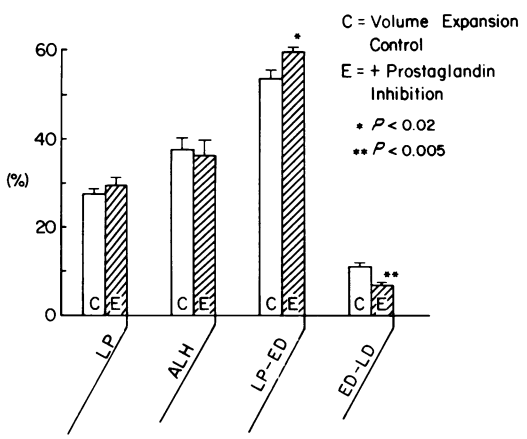

FIGURE 2 Fraction (percent) of filtered chloride reabsorbed along each nephron segment during volume expansion (open bars) and after prostaglandin inhibition (hatched bars). The values at the late proximal (LP) and thin ascending limb of Henle (ALH) represent the fraction of chloride reabsorbed up to these nephron segments. LP–early distal tubule (ED) = fraction of chloride reabsorbed between the LP and ED. ED–LD = fraction of chloride reabsorbed by the distal tubule.

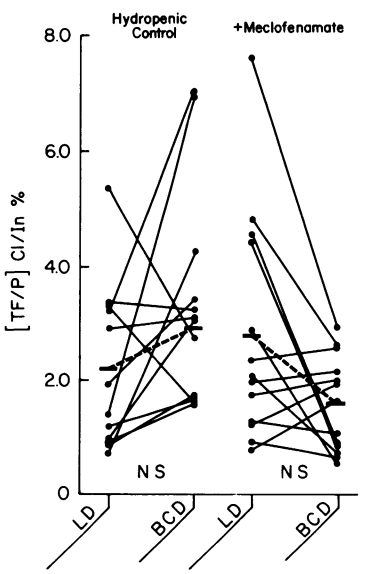

FIGURE 3 Comparison of the fraction of filtered chloride remaining between the late distal tubule (LD) and base of the collecting duct (BCD) during hydropenia and hydropenia after meclofenamate. Each solid line connects a paired sample. The bars connected by the dashed line represents the mean values.

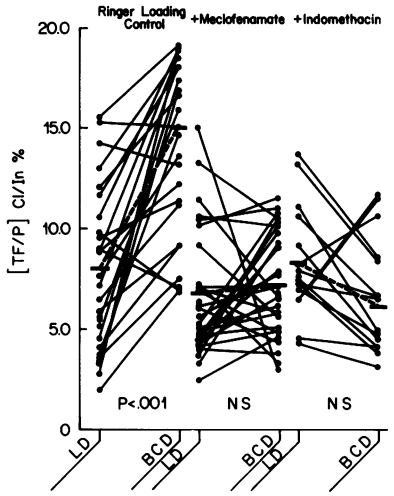

FIGURE 4 Comparison of the fraction of filtered chloride remaining between the late distal tubule (LD) and base of the collecting duct (BCD) during volume expansion and volume expansion plus meclofenamate and indomethacin. Dashed lines connecting bars represent mean values.

DISCUSSION

The present study demonstrates that both indomethacin and meclofenamate, when administered in amounts sufficient to reduce urinary excretion of PGE_2 and $PGF_{2\alpha}$ (Table V), markedly reduce urinary chloride excretion in hydropenic and acutely volume-expanded rats (Table I). Our study, therefore, lends support to previous reports which have advanced evidence that nonsteroidal anti-inflammatory agents result in sodium chloride retention (3–5). The fact that two structurally different nonsteroidal anti-inflammatory drugs had similar effects on chloride transport in each of the segments examined, is evidence against nonspecific, nonprostaglandin-mediated effects.

In this and other studies (10) we have observed that whole kidney, superficial (distal), and juxtamedullary filtration rates were not altered by either of the nonsteroidal antiinflammatory agents used in the present study. Therefore, the representation of chloride reabsorption in fractional terms should also be representative of absolute reabsorption in the segments proximal to the late distal tubule.

That there was no effect of either of these agents on proximal chloride reabsorption is in agreement with other micropuncture studies (11, 12). The significant increase in chloride reabsorption observed between the late proximal and early distal tubule ("short loop of Henle") after indomethacin could be a result of increased reabsorption in either the superficial pars recta, the thin descending limb of Henle, or the thick ascending limb of Henle. It seems most likely that the observed increase in chloride reabsorption occurred in the thick ascending limb. The pars recta would be an unlikely site since the superficial proximal tubule was not affected. The superficial thin descending limb is also an unlikely site for increased reabsorption of chloride since this portion of the nephron appears to lack capacity for active salt transport and the permeability to sodium is exceedingly low (13). In addition, puncture of the thin limb of Henle's loop demonstrated that neither of the drugs had an effect on chloride reabsorption in the deep nephrons up to that point. We are unable to explain completely the observation that the group receiving meclofenamate

TABLE V
Urinary Prostaglandins Measured by Radioimmunoassay

		Urinary concentration		Urinary excretion rate	
		PGE_2	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$
		pg/ml		*pg/min/kg body wt*	
Volume expansion Control	Mean	3,223	9,149	764	2,483
	±SE	578	1,754	164	653
	(*n*)	(7)	(7)	(7)	(7)
Volume expansion + meclofenamate	Mean	338	1,263	47	182
	±SE	40	162	9	37
	(*n*)	(8)	(8)	(8)	(8)
	P	<0.001	<0.001	<0.001	<0.001
Volume expansion + indomethacin	Mean	246	2,741	22	220
	±SE	56	897	4	50
	(*n*)	(5)	(5)	(5)	(5)
	P	<0.005	<0.01	<0.025	<0.025
	*P**	NS	NS	NS	NS

* *P* value compares meclofenamate to indomethacin group.

TABLE VI
Papillary Tissue Chloride Concentration in the Left (Exposed) Papilla

Group		Chloride concentration
		meq/kg tissue H_2O
Hydropenia Control (n = 5)	Mean ±SE	119 8
Hydropenia + meclofenamate (n = 6)	Mean ±SE P	215 24 <0.01
Volume expansion Control (n = 10)	Mean ±SE	127 15
Volume expansion + meclofenamate (n = 8)	Mean ±SE P	181 9 <0.01
Volume expansion + indomethacin (n = 8)	Mean ±SE P P*	220 13 <0.001 <0.05

Each P value obtained by unpaired t test and compares group to either hydropenic or volume expansion controls.
* P value compares volume expansion plus meclofenamate and indomethacin groups.

during volume expansion did not significantly increase fractional chloride reabsorption in the short loop of Henle (Table IV) despite the generation of a steeper transtubular chloride gradient (Table III) and a reduction in early distal chloride delivery. A significant increase in chloride reabsorption in this segment was demonstrated in the group receiving indomethacin (Table IV). It is conceivable that this difference might reflect different effective doses of these two inhibitors of prostaglandin synthesis, or even effects on other regulatory systems. However, chloride reabsorption in the short loop of Henle was not different between these two groups so that these agents, when considered together, had similar effects in this segment.

The observation that these two inhibitors of prostaglandin synthesis increased chloride reabsorption in the superficial loop of Henle, most likely as a result of an increase in chloride transport in the thick ascending limb of Henle, leads us to suspect that for the most part, the reduction in chloride delivery to the base of the collecting duct is a result of an increase in chloride reabsorption in the medullary thick ascending limb of Henle of the juxtamedullary nephrons as well. Furthermore, the evidence supports a direct effect on chloride transport in the medullary thick ascending limb as opposed to a hemodynamic effect. Prostaglandin inhibition has been associated with a decrease in papillary blood flow (14), and thus one could reasonably conclude that such an effect might be occurring in the present study. However, it is unlikely that a reduction in papillary blood flow alone can explain the reduction of fractional chloride delivery found in the early distal tubule and base of the collecting tubule. The evidence for this statement is based on the observed effects of these agents on the papillary interstitial chloride concentration (Table VI). The increase in the concentration of chloride in the papillary interstitium after administration of either meclofenamate or indomethacin as demonstrated in this study and as previously reported by Ganguli et al. (15) could be a result of either decreased papillary blood flow (a decreased "washout") or an increased net NaCl efflux from the medullary portion of the ascending limb of Henle or both. Whatever the mechanism(s) the increased papillary interstitial chloride concentration would create a less favorable gradient for passive chloride reabsorption in the thin ascending limb of Henle and should result in more chloride being delivered out of the juxtamedullary nephrons to the base of the collecting tubule. Thus the combined findings of an increased papillary chloride concentration together with a decreased delivery of chloride out of the juxtamedullary nephrons strongly support the hypothesis that inhibition of prostaglandin production results in an increase in active chloride transport in this segment.

To the extent that these changes represent the effect of a reduction in prostaglandin production, the findings support a role for endogenous prostaglandins in directly inhibiting chloride transport out of the thick asending limb of Henle and/or the cortical and outer medullary collecting tubule. In this regard it is of interest that Stokes (16) has observed that PGE_2 when placed in the bath or perfusate of the isolated in vitro perfused medullary thick ascending limb of Henle of the rabbit, resulted in a significant inhibition of chloride transport and a reduction in transepithelial voltage. These results support our interpretation of the present findings and suggest that endogenous prostaglandins may be having a similar effect in vivo.

Studies in which prostaglandins have been employed directly, as opposed to the use of inhibitors of prostaglandin production, support the findings in the present study. Strandhoy et al. (17) found no effect of prostaglandins on proximal reabsorption when PGE_2 was infused into the renal artery of the dog. Despite this finding, a marked natriuresis was observed. Fulgraff and Meiforth (18) also failed to demonstrate a proximal effect of PGE_2 in the rat whereas the filtered load of sodium delivered to the early distal tubule and the sodium concentration at this site was observed to increase significantly. Using the technique of perfusing

isolated rabbit renal tubules in vitro, Stokes and Kokko (19) have reported that PGE_2 can directly inhibit sodium reabsorption across the cortical and outer medullary collecting tubule. Iino and Imai (20) have confirmed these findings and have also found that $PGF_{2\alpha}$ has similar effects in this segment.

The results of the present study when combined with the studies cited above (16–20) suggest that endogenous renal prostaglandins may play a role in the natriuresis and chloruresis of acute volume expansion by decreasing fractional salt reabsorption in the juxtamedullary nephron preferentially. The majority of the juxtamedullary nephron traverses the renal medulla, an area known to be rich in prostaglandins (21). An increase in medullary prostaglandins in response to volume expansion could directly inhibit chloride reabsorption in the thick ascending limb of Henle. Thus, an increase in medullary prostaglandins could be associated with an increase in the delivery of chloride to the base of the collecting duct. The superficial nephron, on the other hand, might respond somewhat differently. First, because this nephron has only a short transit through the medulla, and does not posses a thin ascending limb, the change in medullary tonicity, which affects water abstraction and passive salt efflux, would have a smaller overall impact. Secondly, the superficial thick ascending limb is composed of both cortical and medullary segments. The cortex represents an area of lower prostaglandin concentration (20) so that the inhibition of chloride transport in this segment might be less marked and would compensate, in part, for a larger chloride delivery eminating from its deeper medullary portion. Furthermore, recent studies in our laboratory cited above (16) have failed to demonstrate a direct effect of PGE_2 on chloride flux or transepithelial potential in the in vitro cortical thick ascending limb of the rabbit whereas a significant decrease in chloride transport was noted in the medullary thick limb. If the results in the rabbit (in vitro) can be extrapolated to the rat (in vivo), both drugs would be expected to increase chloride reabsorption in the medullary, but not the cortical thick ascending limb of Henle. The resultant change in fractional chloride reabsorption in the short loop of Henle, because of its heterogeneous anatomical and functional components, could reflect these differences in precisely the manner demonstrated in this study (Table IV).

In conclusion, our study suggests that inhibition of endogenous prostaglandin production with nonsteroidal antiinflammatory agents results in chloride retention in both hydropenic and volume-expanded rats. The results are compatible with the hypothesis that prostaglandins may directly inhibit chloride reabsorption in the medullary thick ascending limb of Henle but may also inhibit chloride reabsorption in the cortical and outer medullary collecting ducts. Furthermore, the net addition of chloride between the superficial late distal tubule and base of the collecting duct associated with acute volume expansion is blunted by inhibition of endogenous prostaglandins. In this manner, our previous (1), and present findings support an important role for heterogeneous nephron function in the regulation of final sodium chloride excretion.

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