

Collecting Duct Sodium Reabsorption in Deoxycorticosterone-Treated Rats

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ABSTRACT In vitro studies of isolated, perfused, cortical collecting tubules have demonstrated that prior chronic deoxycorticosterone acetate (DOCA) treatment increases sodium reabsorption in this nephron segment, yet sodium balance in vivo is maintained. To evaluate the effect of chronic DOCA treatment on collecting duct sodium reabsorption in vivo, we compared fractional sodium delivery ($FD_{Na}\%$) out of the superficial late distal tubule with the fraction of sodium remaining at the base and the tip of the papillary collecting duct during extracellular fluid volume expansion in untreated, salt-treated, and DOCA-salt-treated rats. In untreated rats, $FD_{Na}\%$ to the distal tubule was $6.5 \pm 1.0\%$, and to the base was $8.7 \pm 1.6\%$ ($\Delta 2.2 \pm 0.9\%$, $P < 0.05$). $FD_{Na}\%$ to the tip was $4.9 \pm 1.1\%$, significantly less than $FD_{Na}\%$ to the base ($\Delta 3.7 \pm 1.1\%$, $P < 0.01$). In salt-treated rats, $FD_{Na}\%$ to the distal tubule was $8.3 \pm 0.8\%$, and to the base was $10.4 \pm 1.1\%$. $FD_{Na}\%$ to the tip was $5.9 \pm 0.6\%$, significantly less than $FD_{Na}\%$ to the base ($\Delta 4.6 \pm 1.0\%$, $P < 0.005$). In DOCA-salt-treated rats, $FD_{Na}\%$ to the distal tubule was $16.1 \pm 2.6\%$ and to the base was $9.5 \pm 1.9\%$ ($\Delta 6.6 \pm 1.7\%$, $P < 0.005$). $FD_{Na}\%$ to the tip was $5.9 \pm 1.2\%$, also significantly less than $FD_{Na}\%$ to the base ($\Delta 3.6 \pm 1.1\%$, $P < 0.01$). We conclude that (a) in DOCA-salt-treated rats, sodium delivery to the end of the superficial distal tubule is greater than in untreated or salt-treated rats; (b) in DOCA-salt-treated rats, sodium delivery to the end of the superficial distal tubule is greater than to the base of the papillary collecting duct, suggesting stimulation of sodium reabsorption in the cortical and(or) outer medullary collecting duct; and (c) sodium reabsorption by the papillary collecting duct is unaffected by chronic DOCA-salt treatment in the volume-expanded rat.

INTRODUCTION

Previous studies with the technique of in vitro perfusion of cortical collecting tubules have shown that this nephron segment is very responsive to mineralocorti-

coids. Gross et al. (1) found that in isolated cortical collecting tubules a significant negative potential difference was found only if the rabbits were pretreated with a low-sodium, high-potassium diet plus deoxycorticosterone acetate (DOCA),¹ or given DOCA alone on a regular diet. O'Neil and Helman (2) reported that cortical collecting tubules from rabbits chronically treated with DOCA had markedly elevated trans-epithelial voltages and increased sodium transport. On the other hand, it is well known that animals return to sodium balance in the presence of mineralocorticoid excess (3), and in rats this DOCA-escape phenomenon occurs after 1.5 d (4). Absolute sodium reabsorption by the papillary collecting duct has been shown to be increased in free-flow micropuncture studies of volume-expanded rats (5, 6). However, the effect of DOCA on this segment has not been previously studied by free-flow micropuncture.

To evaluate collecting duct function in vivo, the present study was undertaken to ascertain by free-flow micropuncture the effect of chronic mineralocorticoid treatment on collecting duct sodium reabsorption. To increase sodium delivery to the collecting system, animals were volume expanded. Cortical collecting duct sodium reabsorption was determined by comparing sodium delivery to the end of superficial late distal tubules with delivery to the papillary base. This comparison was interpreted recognizing that changes in sodium delivery to the base of the papilla could be because of altered contributions of deep nephrons or the medullary collecting duct. Papillary collecting duct sodium reabsorption was determined by comparing differences in sodium delivery to the base and tip of the papilla.

METHODS

Studies were performed on male Munich-Wistar rats that weighed 100–175 g. The animals were anesthetized with

¹ *Abbreviations used in this paper:* DOCA, deoxycorticosterone acetate; $FD_{Na}\%$, fractional delivery of sodium; (TF/P)_{Na/In}, tubule fluid: plasma concentration ratio of Na divided by that of inulin.

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Inactin (100 mg/kg body wt; Byk Gulden Konstanz, Hamburg, West Germany). After a tracheostomy, catheters were inserted in a jugular vein for infusions and in a carotid artery for withdrawal of blood and for blood pressure monitoring. For collection of urine a catheter was inserted in the base of the bladder with the dome of the bladder ligated. Body temperature was maintained between 36 and 38°C with a thermal regulated micropuncture table. Through a small left subcostal incision the left kidney was immobilized and placed in a holder, and bathed with mineral oil. The papilla was exposed by dissection of the ureter. Although it has been reported that exposure of the renal papilla causes a concentrating defect (7), this defect is controlled because all three groups of animals were treated identically. Animals were primed with 0.5 ml of a 4% inulin in a 0.9% saline solution and the rate of inulin infusion was kept constant at 0.02 ml/min. Simultaneously, a 10% body wt volume expansion with 0.9% saline was infused over a period of 1 h, after which the infusion was altered to match urine volume. After this, papillary collecting ducts were micropunctured with pipettes with tip diameters of 10–15 μ m. In each animal, four tubule fluid collections were made at the base of the papilla (base collections), and four collections were made from the tip of the papilla by the axial introduction of the pipette into a duct of Bellini (tip collection). Then from two to four late distal tubule segments were identified by lissamine green injection and punctured with pipettes 4–7 μ m o.d. as previously described (8).

Three experimental protocols were followed:

Group 1: untreated rats. Rats were fed normal Purina Chow (sodium concentration 0.1 meq/g; Ralston Purina Co., St. Louis, Mo.) up to 15 h before experimentation and had free access to water up to the time of the study.

Group 2: salt-treated rats. Rats were fed normal rat chow and had free access to isotonic saline for drinking water for 3 d before the study.

Group 3: DOCA-salt-treated rats. Rats were given normal rat chow and isotonic saline for drinking water for 3 d. DOCA (15 mg/kg i.m.; Ciba Pharmaceutical Company, Div. of CIBA-GEIGY Corp., Summit, N. J.) was administered daily for 3 d before the acute experiment.

From two to three clearance periods were obtained from the right kidney via the bladder catheter at the same time as the micropuncture samples were being collected. Because it has

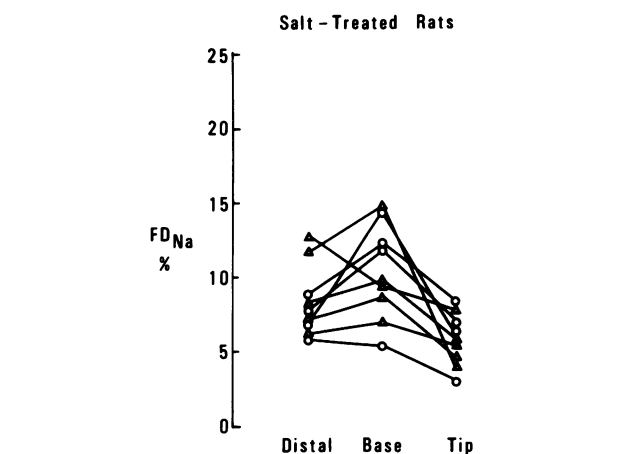


FIGURE 2 Comparison of $FD_{Na}\%$ at the superficial distal tubule, base, and tip of the papilla in salt-treated rats.

been reported that surgical preparation of rats for micropuncture results in a marked decrease in sodium excretion because of a significant reduction of plasma volume (9), five rats in each group were given 1 ml of 25% hyperoncotic albumin after completion of surgical preparation (1 h before micropuncture) and 0.5 ml after blood withdrawal. As can be seen in Figs. 1–3, restoration of plasma volume usually resulted in a higher delivery of sodium, but qualitatively the response was the same as in the animals that did not receive albumin.

Inulin concentrations in plasma and urine were determined by the microfluorometric method of Vurek and Pegram (10). The volume of tubule fluid was measured with micropipettes calibrated with a radioactive tracer. Sodium concentrations in distal and collecting duct fluid were measured with a helium glow photometer. Sodium concentrations in plasma and urine were measured by flame photometry. Comparison of the data within groups was determined by the paired *t* test and between groups by the group *t* test. All results are presented as means \pm 1 SE.

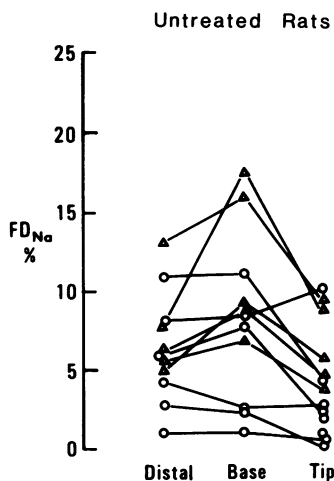


FIGURE 1 Comparison of the $FD_{Na}\%$ at the superficial distal tubule, base of the papilla, and tip of the papilla in untreated rats. Triangles (Δ) represent rats in which plasma volume was repleted with albumin. (see Methods).

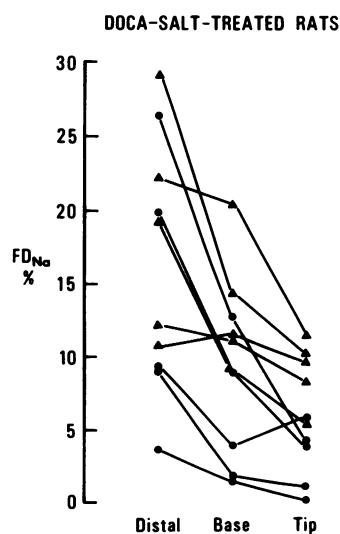


FIGURE 3 Comparison of $FD_{Na}\%$ at the superficial distal tubule, base, and tip of the papilla in DOCA-salt-treated rats.

TABLE I
Summary of Clearance Data for the Contralateral Kidney

Group	Mean arterial blood pressure	GFR*	FE _{Na} %†
	mm Hg	ml/min	%
Untreated rats (n = 11)	122±5§	1.3±0.1	5.5±1.2
Salt-treated rats (n = 9)	135±4	0.8±0.1	6.5±0.9
DOCA-salt-treated rats (n = 10)	128±6	1.2±0.2	6.5±1.6

* Glomerular filtration rate.

† Fractional sodium excretion.

§ Mean±1 SE.

RESULTS

Summaries of the clearances from the contralateral kidney and micropuncture data are shown in Tables I and II.

Group 1: untreated rats. The tubule fluid: plasma concentration ratio of Na divided by that of inulin ($(TF/P)_{Na/In}$) or fractional delivery of sodium ($FD_{Na}\%$) at the superficial late distal tubule was significantly less than $FD_{Na}\%$ to the base of the papilla, $\Delta 2.2\pm0.9\%$, $P < 0.05$. $FD_{Na}\%$ at the tip of the papilla was significantly less than at the base of the papilla, $\Delta 3.7\pm1.1\%$, $P < 0.01$. The single nephron glomerular filtration rate for the collections taken from the superficial late distal tubules averaged 45 ± 5 nl/min.

Group 2: salt-treated rats. The $(TF/P)_{Na/In}$ or $FD_{Na}\%$ at the superficial late distal tubule was $8.3\pm0.8\%$, whereas $FD_{Na}\%$ to the base of the papilla was $10.4\pm1.1\%$. $FD_{Na}\%$ at the tip of the papilla was significantly less than at the base of the papilla, $\Delta 4.6\pm1.0\%$, $P < 0.005$. The mean single nephron glomerular filtration rate for the collections taken from the superficial late distal tubules averaged 49 ± 6 nl/min.

Group 3: DOCA-salt-treated rats. The $FD_{Na}\%$ at the superficial late distal tubule was significantly greater than at the base of the papillary collecting duct, $\Delta 6.6\pm1.7\%$, $P < 0.005$. $FD_{Na}\%$ at the tip of the papilla was also significantly less than at the base, $\Delta 3.6\pm1.1\%$, $P < 0.01$. The mean single nephron glomerular filtration rate for the distal tubule collections was 49 ± 5 nl/min.

Group comparisons between the DOCA-salt-treated rats and the untreated and salt-treated rats revealed three important segmental differences in sodium handling. First, the delivery of sodium at the superficial late distal tubule in the DOCA-salt-treated rats was significantly higher than in the untreated rats, $\Delta 9.6\%$, $P < 0.005$, and in the salt-treated rats, $\Delta 7.8\%$, $P < 0.005$. Second, distal tubule minus papillary base sodium deliveries showed a mean difference Δ of 8.7% , $P < 0.001$, between the untreated and DOCA-salt-treated groups, and a Δ of 8.7% , $P < 0.001$, between the salt-treated and DOCA-salt-treated groups. Third, there was no significant difference between the base and tip of the papilla, $\Delta 0.1\%$, between the untreated and DOCA-salt-treated groups, and $\Delta 1.0\%$, between the salt-treated and DOCA-salt-treated groups.

DISCUSSION

Figs. 1–3 represent the relationship among $FD_{Na}\%$ out of the superficial late distal tubule, base of the papillary collecting duct, and final urinary sodium delivery (tip). First, we will focus on the comparison of $FD_{Na}\%$ at the superficial late distal tubule with the base of the papillary collecting duct. In the untreated animals, there was a significantly higher $FD_{Na}\%$ to the papillary base than to the superficial late distal tubule during volume expansion. In the salt-treated animals, there was a tendency for a higher $FD_{Na}\%$ to the papillary base, although this difference was not statistically significant. These observations are in agreement with

TABLE II
Summary of Micropuncture Studies during 10% Volume Expansion

Model	$(TF/P)_{Na}$			$(TF/P)_{In}$			$(TF/P)_{Na/In} \times 100$		
	Distal	Base	Tip	Distal	Base	Tip	Distal	Base	Tip
Untreated (n = 11)	0.58±.09*	0.80±.08	0.73±.10	8.15±0.89	15.4±3.2	21.4±3.1	6.5±1.0†	8.7±1.6†	4.9±1.1
Salt-treated (n = 9)	0.54±.04	0.99±.09	1.02±.08	6.28±0.63	11.9±1.7	24.0±3.8	8.3±0.8	10.4±1.1†	5.9±0.6
DOCA-salt-treated (n = 10)	0.74±.07	1.10±.06	0.99±.10	6.22±1.60	26.0±9.9	30.9±8.1	16.1±2.6†	9.5±1.9†	5.9±1.2

$(TF/P)_{Na}$, tubule fluid:plasma concentration ratio of Na; $(TF/P)_{In}$, tubule fluid:plasma concentration ratio of inulin.

* Mean±1 SE.

† Statistically significant; paired *t* test, $FD_{Na}\%$.

§ Statistically significant; group *t* test, $FD_{Na}\%$.

a micropuncture study by Stein et al. (6) of the rat papillary collecting duct during volume expansion. These authors suggested that the addition of sodium between the superficial and late distal tubule and the papillary base might be caused by a higher delivery of sodium from the deeper nephrons to the collecting duct during volume expansion. In a subsequent study, direct micropuncture of the deep nephrons during volume expansion confirmed that delivery of sodium to the bend of the loop of Henle in juxtamedullary nephrons was greater than estimates of delivery in the superficial nephrons (11).

In DOCA-salt-treated rats, the $FD_{Na}\%$ to the superficial late distal tubule was significantly higher than delivery to the papillary base. This difference in $FD_{Na}\%$ is consistent with the in vitro observation of enhanced sodium reabsorption of cortical collecting tubules after chronic mineralocorticoid treatment. The enhanced cortical collecting duct sodium reabsorption could be from superficial nephrons (i.e., between the point of micropuncture and the base of the papilla), or in cortical collecting tubules from deep nephrons. These data do not rule out enhanced sodium reabsorption by the medullary collecting duct or other segments of deep nephrons. However, the proximal tubule (12), loop of Henle (13), and distal convoluted tubule (1) have been shown to be relatively mineralocorticoid insensitive. Therefore, in our opinion, the most likely explanation is an enhanced sodium reabsorption by cortical and/or medullary collecting tubules. The marked increase in sodium delivery to the late distal tubule may be the mechanism that accounts for the return to sodium balance in the presence of an increase in sodium reabsorption by the cortical collecting duct. The mechanism responsible for the increased delivery to the late distal tubule is unknown, and further studies will be necessary to determine if this occurs in the absence of superimposed volume expansion.

A second important aspect of the present study is the comparison of $FD_{Na}\%$ at the papillary base with the fractional excretion of sodium (papillary-tip delivery). Sodium was reabsorbed along the papillary collecting duct in the presence of volume expansion, an observation in agreement with several previous free-flow micropuncture studies (5, 6). This is in sharp contrast to the conclusions drawn in two microcatheterization studies by Sonnenberg (14, 15), that either no net reabsorption or even net addition of sodium occurs along the papillary collecting duct during Ringer loading. Important differences in technique, extent of the nephron examined, and experimental protocol between the Sonnenberg studies and the present and previous free-flow micropuncture studies have been noted in a previous paper (6). Sonnenberg (16) also reported papillary collecting duct sodium reabsorption was completely inhibited after DOCA treatment in the rat. It was suggested that the natriuresis of DOCA escape

could be mediated by an inhibition of sodium transport along this nephron segment. However, it is clear from the present study that sodium reabsorption by the terminal portion of the collecting duct is unaffected by chronic DOCA treatment in the volume-expanded rat.

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