

Effects of Uninephrectomy on Electrical Properties of the Cortical Collecting Duct from Rabbit Remnant Kidneys

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Abstract

Microelectrode techniques were used to determine the Na^+ and K^+ transport properties of the collecting duct cell in the isolated cortical collecting duct (CCD) from rabbits 14 d after uninephrectomy (UNX); results were compared with those from sham-operated rabbits (control). UNX had no effects on plasma aldosterone levels. The CCDs from UNX rabbits exhibited structural hypertrophy. The lumen negative transepithelial voltage and the basolateral membrane voltage (V_B) were elevated in the UNX group. Although the transepithelial conductance (G_T) and the fractional apical membrane resistance (fR_A) were not different between the two groups, the conductances of the apical and the basolateral membranes were increased, and the tight junction conductance was decreased in the UNX group. The amiloride-sensitive changes in apical membrane voltage (V_A), fR_A , and G_T were greater in the UNX group. The changes in V_A upon raising the perfusate K^+ concentration and the changes in V_A and G_T upon addition of Ba^{2+} to the perfusate were elevated in the UNX group. Upon raising K^+ in the bath, a large depolarization of V_B was observed in the UNX group. Lowering the bath Cl^- resulted in a small depolarization of V_B in the UNX group. Addition of Ba^{2+} to the bath in the UNX group caused the V_B to hyperpolarize in parallel with decreases in G_T and fR_A whereas in the control group it had no effect on V_B . Addition of ouabain to the bath resulted in a large depolarization of V_B in the UNX group. We conclude that (a) UNX stimulates conductances of Na^+ and K^+ in the apical membrane, active Na^+-K^+ pump activity, and K^+ conductance in the basolateral membrane, independently of plasma aldosterone; (b) The basolateral membrane in the tubules of UNX rabbits is more selective to K^+ ; and (c) the hyperpolarization of V_B upon UNX may increase passive K^+ entry into the cell across the basolateral membrane. (*J. Clin. Invest.* 1992. 90:1547-1557.) Key words: electrophysiology • distal delivery of sodium • sodium conductance • potassium conductance • sodium pump

Portions of this work have been published in abstract form (1991. FASEB [*Fed. Am. Soc. Exp. Biol.*] J. 5:739A).

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Received for publication 21 October 1991 and in revised form 13 May 1992.

J. Clin. Invest.

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0021-9738/92/10/1547/11 \$2.00

Volume 90, October 1992, 1547-1557

Introduction

After loss of renal mass, the remaining nephrons, including the cortical collecting duct (CCD),¹ undergo a compensatory hypertrophy and an adaptation that maintains water, electrolytes, and acid-base balance (1-3). For electrolyte balance, previous studies of the CCDs of remnant kidneys after nephrectomy exhibited adaptive increase in Na^+ reabsorption (4) and K^+ secretion (5). These adaptive changes also included an increase in Na^+/K^+ ATPase activity (6, 7) and an amplification of the basolateral membrane area of the collecting duct (CD) cell (8). These findings suggest that the CD cell, which is mainly responsible for Na^+ and K^+ transports in the CCD (9-16), would play the key role in the adaptation after reduction of renal mass. However, the cellular mechanisms of these adaptive changes remain unknown. The purpose of the present study was to determine the electrical properties in the apical as well as the basolateral membranes of the CD cell in the CCD from uninephrectomized rabbits.

Chronic elevations in aldosterone levels are also known to stimulate the proliferation of the basolateral membrane area of the CD cell (17), to enhance Na^+/K^+ ATPase activity (18, 19) and to increase the Na^+ and K^+ transport capacity in the CCD (11, 16, 20, 21). Thus, elevated aldosterone levels would induce the functional and morphological adaptations in the CCD from remnant kidneys after nephrectomy. Therefore, we also determined whether the changes in plasma aldosterone contribute to the adaptations in the CCD after a reduction of renal mass.

The present study demonstrates that uninephrectomy stimulates conductances of Na^+ and K^+ in the apical membrane, active Na^+/K^+ ATPase pump activity, and K^+ conductance in the basolateral membrane, independently of plasma aldosterone. These functional adaptive changes are associated with structural hypertrophy in the CCD from remnant kidneys.

Methods

Animals and surgical procedures. Female Japanese white rabbits were used for all experiments. After a period of acclimation, the rabbits were divided into two groups: sham operation (control) and uninephrec-

1. *Abbreviations used in this paper:* CCD, cortical collecting duct; CD cell, collecting duct cell; E_B^K , Nernst equilibrium potential for K^+ across the basolateral membrane; ΔEMF , change in basolateral electromotive force due to ion substitution; fR_A , fractional apical membrane resistance; G_A , apical membrane conductance; G_B , basolateral membrane conductance; G_T , transepithelial conductance; G_{Tj} , tight junction conductance; uninephrectomy, UNX; V_A , apical membrane voltage; V_B , basolateral membrane voltage; V_T , transepithelial voltage.

tomy (UNX). The operative procedures were as follows: First, all the animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Second, the abdomen of each animal was opened by a left flank incision. After the abdomen was opened, the left kidney of those in the UNX group was removed via a dorsolateral incision under sterile conditions. The kidney was separated from the adrenal gland and from the associated connective tissue, the renal blood vessels were ligated, and the organ was excised. Then, the abdominal musculature in both groups of animals was sewn. The rabbits from two groups were maintained on standard rabbit chow (rabbit chow, Clea Japan, Inc., Tokyo, Japan), containing Na⁺ of 120 meq/kg diet and K⁺ of 400 meq/kg diet, and tap water ad lib.

Metabolic balance studies. Balance studies were performed on two groups of animals. On the 14th day after surgery, they were individually placed in the metabolic cages for 24 h. During these periods, Na⁺, K⁺, Cl⁻, and creatinine concentrations in the plasma and in the urine were measured. Also, the urine volume for 24 h was determined gravimetrically. The GFR was measured in terms of the endogenous creatinine clearance. The creatinine clearance was calculated by standard formulas using urine/plasma concentration ratios and urine flows.

Isolation and perfusion of tubules. On the 15th day after surgical procedure, the animals of both groups were anesthetized with sodium pentobarbital (35 mg/kg, i.v.), the right kidney was removed and weighed. Slices of the coronary section 1–2-mm thick were made and transferred to a dish containing a cold intracellular fluid-like solution of the following composition (mM): 14 KCl, 44 K₂HPO₄, 14 KH₂PO₄, 9 NaHCO₃, and 160 sucrose. The CCD segments were isolated and perfused in vitro according to the method of Burg et al. (22) with slight modifications in this laboratory (13, 14). Briefly, the tubule segment was perfused via a perfusion pipette inserted into one end of the tubule. A PE-10 tube (Intramedic; Clay Adams, Parsippany, NJ) was inserted into the perfusion pipette to permit rapid exchange of the perfusate with a test solution within 5 s. The opposite or distal end of the tubule was held in a glass holding pipette with a small amount of Sylgard 184 (Dow Corning Corp., Midland, MI). The perfusion flow rates were > 20 nl/min and controlled by the height of the outflow sink. The tubule was perfused in the bathing chamber of ~ 100 μl to permit rapid exchange of the bathing solution within 5 s. The bathing solution flowed at 5–15 ml/min from the reservoirs by gravity through a water jacket to permit the bath temperature to be regulated at 37°C.

Electrical measurements. The transepithelial and cellular electrical properties of the tubule were measured using techniques described previously by Muto et al. (13, 14, 16). In brief, the transepithelial voltage (V_T) was measured through the perfusion pipette, which was connected to one channel of a dual-channel electrometer (Duo 773; W-P Instruments, Inc., New Haven, CT) with a 3 M KCl–3% agar bridge and a calomel half-cell electrode. The basolateral membrane voltage (V_B) was measured with 0.5 M KCl-filled microelectrodes, which were fabricated from borosilicate glass capillaries (GD-1.5; 1.5 mm OD, 1.0 mm ID; Narishige Scientific Laboratory, Tokyo, Japan) by using a vertical puller (PE-2; Narishige Scientific Laboratory). Both voltages were referenced to the bath and were recorded on a four-pen chart recorder (R64; Rikadenki, Tokyo, Japan). Cable analysis was used to calculate the transepithelial conductance (G_T), and the fractional apical membrane resistance (fR_A) as described in detail previously (9, 10, 13–16). Constant-current pulses, 50 nA (300 ms in duration, 10-s interval), were injected into the tubule lumen via the perfusion pipette. The fR_A was calculated from the deflection of the apical membrane voltage (V_A) with current passage, divided by the change in the V_T calculated for the point of impalement.

The basolateral, apical, and tight junction conductances (G_B , G_A , and G_{Tj} , respectively) were estimated using 2 mM Ba²⁺ as a probe to the equation described previously by O'Neil and Sansom (10) and Muto et al. (16): $G_T = (1 - fR_A)G_B + G_{Tj}$.

Ion substitution studies were conducted to determine the relative ion permselectivity of the basolateral membrane. When the ion concentration of the bathing solutions was changed, the initial peak change of V_B was used with fast bath exchange rates (2–5 s) to minimize sec-

ondary effects such as changes in cellular ion activities. Voltage changes due to lowering bath Cl⁻ and raising bath K⁺ concentrations were corrected for liquid junction potentials with free-flowing 3 M KCl electrodes. The change in basolateral membrane electromotive force due to ion substitution (ΔEMF) was estimated according to the following equation: $\Delta EMF = \Delta V_B - I \cdot R_B$ where ΔV_B is the measured change in V_B due to ion substitution and $I \cdot R_B$ is the change in membrane potential due to the dissipation of energy from current flowing across the basolateral membrane resistance. The circular loop current (I) was then estimated from G_{Tj} and the change in V_T on ion substitution (ΔV_T) as $I = G_{Tj} \cdot \Delta V_T$.

Identification of CD cells. Electrical identification of CD cells was performed according to the criteria described previously by Muto et al. (13–16). CD cells have a relatively lower fR_A ; higher V_B , apical Na⁺, and K⁺ conductances; and basolateral K⁺ and Cl⁻ conductances.

Solutions and materials. The composition of the control bathing and perfusing solution was as follows (mM): 110 NaCl, 5 KCl, 1 MgCl₂, 1.8 CaCl₂, 25 NaHCO₃, 10 Na acetate, 0.8 Na₂HPO₄, 0.2 NaH₂PO₄, 5 L-alanine, and 8.3 D-glucose. This control solution had an osmolality between 285 and 295 mosmol/kg/H₂O, and was equilibrated with 95% O₂/5% CO₂ and adjusted to pH 7.4 at 37°C. In some experiments, 45 mM Na⁺ was replaced with K⁺, or 108.6 mM Cl⁻ was replaced with cyclamate.

Amiloride (Sigma Chemical Co., St. Louis, MO) was added to the luminal perfusate to achieve a final concentration of 50 μM. Ouabain (Sigma Chemical Co.) was used in the bath at a concentration of 10⁻⁴ M. BaCl₂ was used at a final concentration of 2 mM.

Tubular measurements. Tubular lengths were measured at the end of each experiment with a calibrated reticle in the eyepiece of the microscope. The tubules were photographed during perfusion at a proximal, central, and distal site at a magnification of 200. Inner and outer diameters were measured at 0.05-mm intervals along the tubule. Reported values are the average of at least five measurements. Since the tubules from both groups were rapidly perfused at similar rates and pressures, the degree of distention of the lumen is assumed to be similar in all.

Radioimmunoassay for plasma aldosterone. At 16 h and 7 and 14 d after the surgery, blood was also taken from control and UNX rabbits. Specific radioimmunoassay (SPAC-S Aldosterone Kit; Daiichi Radio Isotope Labs, Tokyo, Japan) was used to measure the plasma aldosterone concentration.

Statistics. The data are expressed as mean ± SE. Differences between groups were determined by the Student's *t* test for either paired or nonpaired data as appropriate. *P* values < 0.05 were considered significant.

Results

Effects of UNX on body and kidney weights

Body and kidney weights in control and UNX animals are given in Table I. UNX had no adverse effects on body growth as UNX rabbits attained the same weight at 14 d as did control

Table I. Effects of Uninephrectomy on Body and Kidney Weights

Group	Body weight		Right kidney weight
	initial	final (day 14)	
	kg		g
Control (n = 20)	1.99 ± 0.04	2.32 ± 0.04	7.5 ± 0.3
UNX (n = 24)	1.97 ± 0.04	2.23 ± 0.04	11.3 ± 0.4
<i>P</i>	NS	NS	<0.005

Values are mean ± SE. *n*, number of animals.

Table II. Metabolic Balance in Control and UNX Rabbits

Group	Plasma				Urine volume ml/d	Daily urinary excretion			
	Na	K	Cl	Cr		Na	K	Cl	Cr
	meq/liter	meq/liter	meq/liter	mg/dl		meq/d	meq/d	meq/d	mg/d
Control (n = 13)	138.4±0.9	4.3±0.1	97.5±0.8	0.9±0.1	163.6±16.9	9.2±2.5	39.1±4.6	15.8±2.3	103.8±11.9
UNX (n = 11)	137.8±1.6	4.3±0.2	97.8±1.4	0.9±0.1	172.5±19.6	9.5±1.2	40.0±5.7	16.2±2.6	99.2±16.7

Values are mean±SE. Cr, creatinine; n, number of animals.

rabbits. The kidney growth, however, was significantly altered, with the remaining right kidney undergoing compensatory hypertrophy.

Metabolic balance study

Table II shows plasma electrolytes and creatinine concentrations and their daily urinary excretions in control and UNX rabbits. UNX had no significant effects on plasma creatinine concentration and its daily urinary excretion. In addition, there were also no significant differences of plasma levels of electrolytes and their daily urinary excretions between the two groups.

Renal function

14 d after UNX, the GFR was significantly greater in the hypertrophied remaining kidney from the UNX rabbits (8.2±1.5 ml/min per kidney, n = 11, P < 0.025) than in the kidney from the control rabbits (4.6±0.6 ml/min per kidney, n = 13). This increase was paralleled by both a significantly higher absolute sodium reabsorption rate (1,127.3±201.6 vs. 627.1±86.6 μeq/min per kidney, P < 0.025) and a significantly higher absolute potassium excretion rate (27.8±4.0 vs. 13.6±1.6 μeq/min per kidney, P < 0.001) in the hypertrophied remaining kidney. These findings are similar to those reported by Mujais and Kurzman (7) in the UNX rats 14 d after surgery.

Electrophysiological data

The length of the perfused tubule in the control and UNX groups was 1,007.2±48.2 (n = 32) and 1,052.8±81.0 μm (n = 18), respectively. On the other hand, both the inner and outer diameters of 18 tubules from UNX rabbits were significantly greater than those of 32 tubules from control rabbits (inner diameters: 33.0±1.1 vs. 29.3±0.8 μm, P < 0.01; outer diameters: 42.8±1.1 vs. 39.1±0.7 μm, P < 0.01). These results

indicate the compensatory hypertrophy of CCDs from UNX rabbits. These findings are consistent with those reported by Vehaskari et al. (4), in the CCD from the remnant kidneys of the rabbits following surgical reduction of renal mass by 3/4 to 7/8.

Effects of UNX on barrier voltages and conductances. The potential profiles of CD cells of tubules of the two groups are illustrated in Fig. 1. The V_T and V_B of the tubules from UNX rabbits were significantly higher than those of the tubules from control rabbits. On the other hand, the calculated V_A was unchanged upon UNX (75.0±1.8 mV vs. 80.7±2.6 mV).

Table III shows the effects of UNX on fR_A and barrier conductances. The values of fR_A and G_T were not significantly different between the two groups. However, both G_A and G_B were significantly higher and G_{Tj} was significantly lower in the UNX group.

Effects of UNX on electrical properties of the apical membrane. As described above, UNX produced a 2.5-fold increase in G_A . The first set of the studies was, therefore, designed to examine whether this increase in G_A is the result of a change in the Na^+ conductance and/or K^+ conductance in the apical membrane of the CD cell.

To examine whether a difference in Na^+ conductance of the apical membrane of the CD cell exists between the two groups, we added a Na^+ channel inhibitor, amiloride, to the perfusate and observed the barrier voltages and conductances. Typical tracings of V_T and V_B in the tubules of two groups upon addition of 50 μM amiloride to the perfusate are illustrated in Fig. 2. The effects of luminal amiloride on barrier voltages and conductances in the two groups are summarized in Table IV. Upon addition of luminal amiloride, the V_T and the V_B in the tubules of both groups were rapidly depolarized, resulting in a significant hyperpolarization of V_A . However, the amiloride-sensitive changes in V_A were significantly greater in the UNX

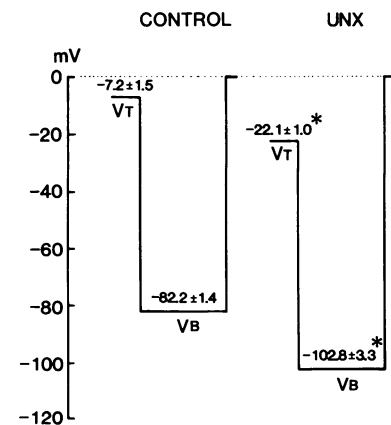


Figure 1. Potential profiles of CD cells from CCDs of CONTROL and UNX rabbits. Values are mean±SE. Number of experiments in each group was 24. *P < 0.001 compared with the control group.

Table III. Effects of Uninephrectomy on Fractional Apical Membrane Resistance and Barrier Conductances

	fR_A	G_T	G_A	G_B	G_{Tj}
	mS·cm ⁻²				
Control (n = 12)	0.38±0.03	8.7±0.6	13.4±2.1	7.8±1.2	4.0±0.4
UNX (n = 17)	0.34±0.04	10.8±0.8	33.9±5.9	13.0±1.4	2.7±0.3
P	NS	NS	<0.01	<0.05	<0.05

Values are mean±SE.

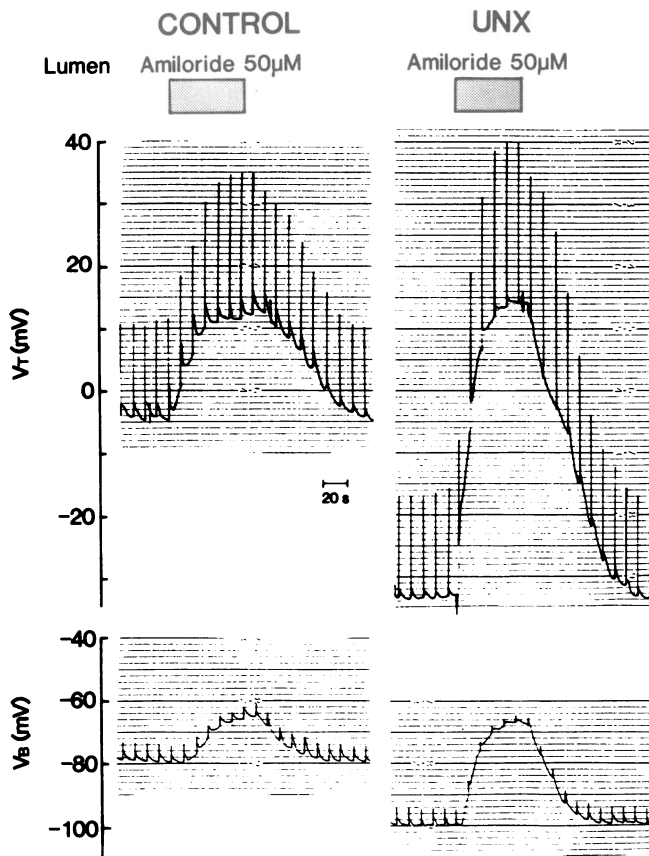


Figure 2. Typical tracings of V_T and V_B upon addition of $50 \mu\text{M}$ amiloride to the perfusate in the tubules of CONTROL and UNX groups.

group. When the amiloride was added to the luminal perfusate in the tubules of the control group, the G_T was significantly decreased, and the fR_A was significantly increased. Although a similar pattern of the responses of G_T and fR_A was also observed in the tubules of the UNX group, the changes in G_T and fR_A were significantly greater. Taken together, these results indicate that the amiloride-sensitive Na^+ conductance in the apical membrane of the CD cell from UNX rabbits is significantly elevated.

The next set of studies was designed to examine whether the apical membrane K^+ conductance of the CD cell in the tubules from UNX rabbits is increased. We compared the electrical properties between the two groups upon raising the perfusate K^+ concentration from 5 to 50 mM. Typical tracings of V_T and V_B in the tubules of both groups are illustrated in Fig. 3. The effects of raising the perfusate K^+ concentration on barrier voltages and conductances at the initial peak response are summarized in Table V. When the perfusate K^+ concentration was increased in the tubules of both groups, the lumen-negative V_T was rapidly increased within several seconds and was then slowly decreased. The V_B was also rapidly depolarized within several seconds and was then slowly further depolarized to a new steady state. During this fast phase, the V_A was rapidly depolarized in both groups. However, the initial peak changes in V_A were significantly greater in the UNX group. Upon raising the luminal perfusate K^+ concentration in the tubules of the control group, the G_T was significantly increased and the fR_A was significantly decreased. Similar pattern of the responses of G_T and fR_A was also observed in the tubules of the UNX group. However, the changes in G_T were greater in the UNX group. These results indicate that the apical membrane K^+ conductance is also increased upon UNX.

To further characterize the apical membrane K^+ conductive property after nephrectomy, we added a K^+ channel inhibitor, Ba^{2+} , to the perfusate, and observed the electrical properties. Typical tracings of V_T and V_B upon addition of luminal Ba^{2+} are illustrated in Fig. 4. The effects of Ba^{2+} to the perfusate on barrier voltages and conductances at the initial peak response are summarized in Table VI. When 2 mM Ba^{2+} was added to the perfusate in the tubules of both groups, the lumen-negative V_T was rapidly increased and was then slowly decreased to a new steady state. The V_B was rapidly depolarized within several seconds and was then slowly depolarized further to a new steady state. These findings are compatible with those reported by Sansom and O'Neil (23) and Muto et al. (16) in CCDs from normal rabbits. This fast phase is considered to represent the initial block of apical membrane K^+ conductance and current (16, 23). During this fast phase, the V_A was rapidly depolarized in both groups. However, the initial peak changes in V_A were much greater in the UNX group. Upon addition of Ba^{2+} to the perfusate in the tubules of the control group, the G_T

Table IV. Effects of $50 \mu\text{M}$ Amiloride in the Lumen on Barrier Voltages and Conductances

	V_T	V_B	V_A	G_T	fR_A
	mV	mV	mV	$\text{mS}\cdot\text{cm}^{-2}$	
Control					
Without amiloride	-7.6 ± 1.4 (18)	-79.8 ± 1.6 (18)	72.2 ± 1.9 (18)	8.9 ± 1.2 (8)	0.50 ± 0.09 (8)
With amiloride	5.4 ± 1.2 (18)	-72.1 ± 2.0 (18)	77.6 ± 2.0 (18)	7.2 ± 1.1 (8)	0.66 ± 0.09 (8)
Difference	13.0 ± 1.4	7.7 ± 0.9	5.3 ± 0.9	-1.4 ± 0.4	0.16 ± 0.03
P	<0.001	<0.001	<0.001	<0.001	<0.005
UNX					
Without amiloride	-29.0 ± 0.9 (14)	-101.1 ± 1.9 (14)	72.0 ± 1.7 (14)	10.4 ± 0.9 (11)	0.44 ± 0.05 (11)
With amiloride	9.6 ± 2.0 (14)	-71.5 ± 2.3 (14)	81.1 ± 1.4 (14)	6.5 ± 0.3 (11)	0.72 ± 0.02 (11)
Difference	$38.6 \pm 2.5^*$	$29.3 \pm 2.5^*$	$10.7 \pm 0.8^*$	$-3.9 \pm 0.9^{**}$	$0.28 \pm 0.04^{**}$
P	<0.001	<0.001	<0.001	<0.005	<0.001

Values are mean \pm SE. Numerals in parentheses indicate number of experiments. * $P < 0.001$ vs. control; ** $P < 0.05$. Data were obtained from the same experiments.

Table V. Effects of Luminal K^+ Elevation from 5 to 50 mM on Barrier Voltages and Conductances

	V_T	V_B	V_A	G_T	fR_A
	mV	mV	mV	$mS \cdot cm^{-2}$	
Control					
K^+ , 5 mM	-6.3 ± 0.9 (22)	-82.4 ± 1.5 (22)	76.2 ± 1.6 (22)	9.5 ± 1.0 (8)	0.47 ± 0.07 (8)
K^+ , 50 mM	-24.9 ± 2.5 (22)	-55.1 ± 2.8 (22)	30.2 ± 2.0 (22)	16.7 ± 3.2 (8)	0.33 ± 0.07 (8)
Difference	-18.4 ± 2.6	27.3 ± 2.7	-46.0 ± 2.0	7.1 ± 2.6	-0.14 ± 0.04
P	<0.001	<0.001	<0.001	<0.05	<0.05
UNX					
K^+ , 5 mM	-25.0 ± 1.5 (18)	-103.7 ± 2.2 (18)	78.5 ± 2.1 (18)	11.4 ± 1.0 (9)	0.38 ± 0.05 (9)
K^+ , 50 mM	-53.5 ± 2.1 (18)	-76.0 ± 3.2 (18)	22.6 ± 3.2 (18)	32.1 ± 5.2 (9)	0.17 ± 0.06 (9)
Difference	$-28.5 \pm 1.9^*$	27.7 ± 2.3	$-55.9 \pm 2.8^*$	$20.7 \pm 4.5^{**}$	-0.21 ± 0.02
P	<0.001	<0.001	<0.001	<0.005	<0.001

Values are mean \pm SE. Numerals in parentheses indicate number of experiments. * $P < 0.01$ vs. control; ** $P < 0.05$. Data were obtained from the same experiments.

was significantly decreased and the fR_A was significantly increased. Although the pattern of the responses of G_T and fR_A in the UNX group was the same as that in the control group, the changes in G_T were much greater in the UNX group. These

results indicate that Ba^{2+} -sensitive K^+ conductance in the apical membrane of the CD cell in the tubules from UNX rabbits is also stimulated.

Effects of UNX on electrical properties of the basolateral membrane. As summarized in Table III, the tubules from UNX rabbits had a 1.6-fold increase in G_B . We next examined whether this increase in G_B is due to either an increase in K^+ conductance and/or an increase in Cl^- conductance in the basolateral membrane. Therefore, we assessed the relative conductive properties of the basolateral membrane of both groups using rapid bath exchange rates. Typical tracings of V_B in the tubules of both groups upon changing bath Cl^- and K^+ concentrations are illustrated in Fig. 5. When bath Cl^- concentration was decreased from 120.6 to 12 mM, the V_B in the tubules from both groups was rapidly depolarized within several seconds and was then repolarized to a new steady state level. Although the pattern of the response was the same in both groups, the magnitude of the initial peak depolarization of the V_B was significantly lower in the UNX group (23.3 ± 2.8 mV, $n = 15$, $P < 0.05$) than in the control group (28.3 ± 1.6 mV, $n = 16$). At that time, the change in electromotive force (ΔEMF) was also significantly lower in the UNX group (25.8 ± 2.1 vs. 35.7 ± 2.2 mV, $P < 0.01$). When bath K^+ concentration was increased from 5 to 50 mM, the V_B in the tubules of the control group was rapidly depolarized within several seconds and was then slowly depolarized further over the next 30 s. Similar pattern of the response of V_B was also observed in the tubules of the UNX group. However, the initial peak changes in V_B were significantly greater in the UNX group (35.1 ± 1.9 mV, $n = 25$, $P < 0.001$) when compared with the control group (17.8 ± 1.3 mV, $n = 22$). The ΔEMF upon raising bath K^+ was also significantly greater in the UNX group (30.9 ± 1.5 vs. 10.4 ± 0.9 mV, $P < 0.001$). These findings indicate that the basolateral membrane of the CD cell in the CCDs from the UNX rabbits is more selective to K^+ .

Table VII shows the effects of raising the bath K^+ concentration on barrier voltages and conductances at the initial peak response. When the bath K^+ concentration was increased in the tubules of both groups, the G_T and the fR_A were significantly increased. However, the changes in G_T and the fR_A were amplified in the UNX group. Taken together, these results indicate that the basolateral membrane K^+ conductance is stimulated upon UNX.

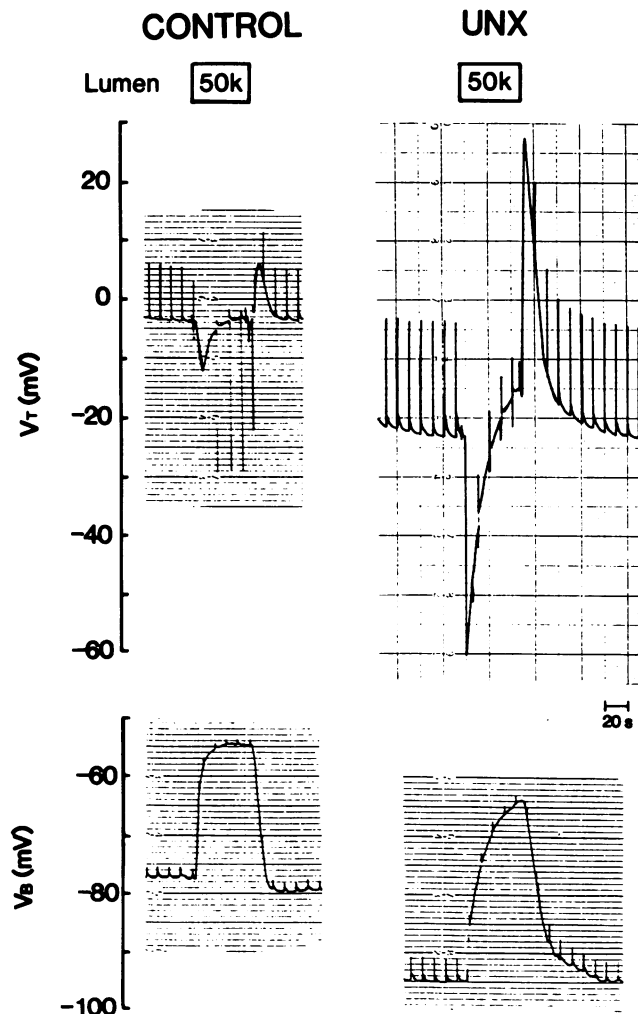


Figure 3. Typical tracings of V_T and V_B upon raising the perfusate K^+ concentration from 5 to 50 mM in the tubules of CONTROL and UNX groups.

Table VI. Effects of 2 mM Ba²⁺ in the Lumen on Barrier Voltages and Conductances

	V _T	V _B	V _A	G _T	fR _A
	mV	mV	mV	mS·cm ⁻²	
Control					
Without Ba ²⁺	-9.3±2.0 (12)	-83.4±2.1 (12)	74.1±2.4 (12)	8.7±0.6 (12)	0.38±0.03 (12)
With Ba ²⁺	-16.7±2.2 (12)	-62.3±1.7 (12)	45.6±3.0 (12)	5.3±0.4 (12)	0.83±0.02 (12)
Difference	-7.2±0.8	21.1±2.0	-28.4±2.4	-3.4±0.4	0.45±0.02
P	<0.001	<0.001	<0.001	<0.001	<0.001
UNX					
Without Ba ²⁺	-24.9±1.2 (17)	-97.2±1.9 (17)	72.2±1.4 (17)	10.9±0.8 (17)	0.34±0.04 (17)
With Ba ²⁺	-40.4±1.8 (17)	-77.2±2.8 (17)	36.8±2.4 (17)	5.0±0.3 (17)	0.80±0.01 (17)
Difference	-15.4±0.9*	20.0±1.4	-35.4±1.5**	-5.8±0.7**	0.46±0.04
P	<0.001	<0.001	<0.001	<0.001	<0.001

Values are mean±SE. Numerals in parentheses include number of experiments. * P < 0.001 vs. control; ** P < 0.05. Data were obtained from the same experiments.

To further characterize the basolateral membrane K⁺ conductive property in the UNX group, we added 2 mM Ba²⁺ to the bath. A typical tracing of V_T and V_B in the UNX group is illustrated in Fig. 6. The effects of the addition of Ba²⁺ to the bath on the electrical properties at the initial peak response are summarized in Table VIII. In the control group, addition of Ba²⁺ to the bath had no significant effects on V_T and V_B, al-

though it caused both G_T and fR_A to decrease significantly (Table VIII). These findings indicate that K⁺ is close to equilibrium across the basolateral membrane. These results are in good agreement with those reported by Sansom and O'Neil (24), in CCDs from normal rabbits. In sharp contrast to the control group, when 2 mM Ba²⁺ was added to the UNX group, V_T and V_B were rapidly hyperpolarized within several seconds by ~ 4 mV in parallel with decreases in G_T and fR_A. These results are consistent with Ba²⁺ blockade of a K⁺ current directed into the cell from the bath in the UNX group. These findings are also observed in the CCDs from desoxycorticosterone acetate-treated rabbits (24).

As shown in Fig. 1, the -V_B was increased by ~ 20 mV in the tubules from UNX rabbits, suggesting that UNX stimulates the Na⁺/K⁺ ATPase pump activity in the basolateral membrane of the CD cell. To further confirm this proposal, we added 10⁻⁴ M ouabain to the bath, and observed the electrical properties. Typical tracings of V_B in the tubules of both groups are illustrated in Fig. 7. In the control group addition of ouabain to the bath resulted in a two-phase depolarization of the basolateral membrane, with an initial rapid depolarization followed by a slow and more prolonged phase. These findings are similar to those reported by Koeppen et al. (9) and Sansom and O'Neil (24) in rabbit CCD, and by Helman et al. (25) in frog skin. This initial fast-depolarizing phase has been attributed to direct inhibition of an electrogenic Na⁺-K⁺ pump, since during this fast-depolarizing phase there was no significant effects on G_T or fR_A (Table IX). Similar pattern of responses of V_B upon addition of bath ouabain was also observed in the UNX group. However, the initial peak changes in V_B were significantly higher in the UNX group, as shown in Table IX. These results are consistent with the view that the Na⁺/K⁺ ATPase pump activity in the tubules from UNX rabbits is also stimulated. This is also supported by the evidence that the Na⁺/K⁺ ATPase activity of the CCDs from the remnant kidneys of the rabbits 14 d after the surgery was stimulated (6).

Effects of UNX on plasma aldosterone concentration

The results described above are quite similar to those seen in the CCDs from chronic DOCA-treated rabbits (9, 23, 24, 26). To examine whether the electrical properties in the apical as well as the basolateral membranes of the CD cell in the CCDs from UNX rabbits are the result of an increased plasma aldoste-

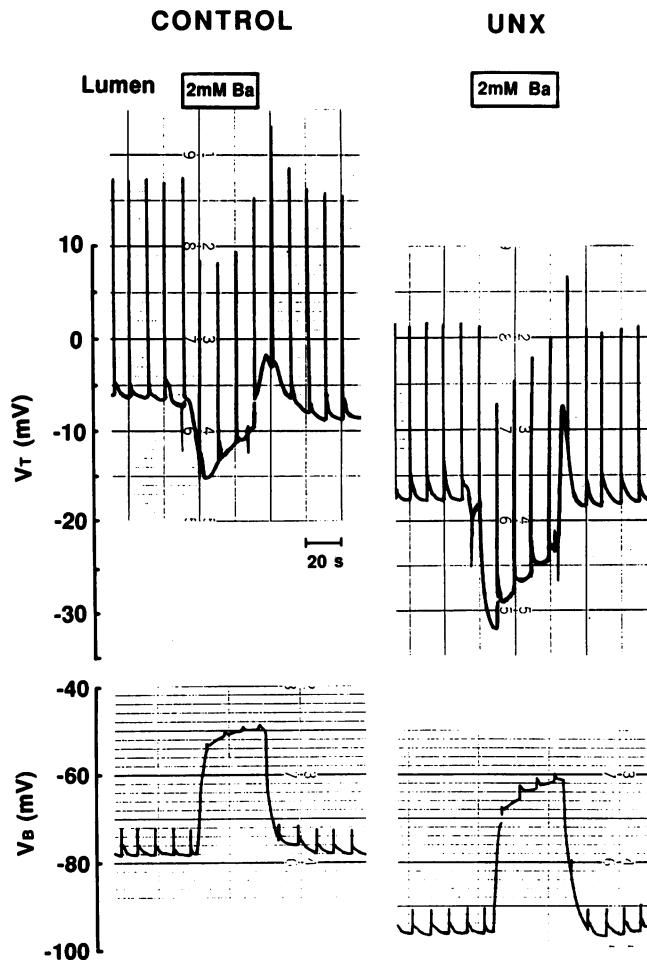


Figure 4. Typical tracings of V_T and V_B upon addition of 2 mM Ba²⁺ to the perfusate in the tubules of CONTROL and UNX groups.

Table VII. Effects of Bath K^+ Elevation from 5 to 50 mM on Barrier Voltages and Conductances

	V_T	V_B	G_T	fR_A
	mV	mV	$mS \cdot cm^{-2}$	
Control				
K^+ , 5 mM	-5.5 ± 1.4 (22)	-80.8 ± 1.8 (22)	9.0 ± 0.7 (9)	0.33 ± 0.07 (9)
K^+ , 50 mM	9.6 ± 1.5 (22)	-63.0 ± 1.8 (22)	10.6 ± 1.0 (9)	0.43 ± 0.08 (9)
Difference	16.2 ± 1.1	17.8 ± 1.3	1.6 ± 0.6	0.10 ± 0.01
<i>P</i>	<0.001	<0.001	<0.05	<0.001
UNX				
K^+ , 5 mM	-21.3 ± 1.2 (25)	-98.4 ± 2.1 (25)	11.3 ± 1.3 (10)	0.21 ± 0.04 (10)
K^+ , 50 mM	11.5 ± 1.1 (25)	-63.2 ± 2.4 (25)	17.9 ± 2.3 (10)	0.40 ± 0.05 (10)
Difference	$32.7 \pm 1.3^*$	$35.1 \pm 1.9^*$	$6.6 \pm 1.1^{**}$	$0.18 \pm 0.03^{**}$
<i>P</i>	<0.001	<0.001	<0.001	<0.001

Values are mean \pm SE. Numerals in parentheses indicate number of experiments. * $P < 0.001$ vs. control; ** $P < 0.05$. Data were obtained from the same experiments.

rone concentration, we measured plasma aldosterone levels at 16 h and 7 and 14 d after surgery in the two groups. Plasma aldosterone levels at 16 h and 7 and 14 d in the UNX rabbits were 66.0 ± 8.1 ($n = 6$), 71.0 ± 8.9 ($n = 5$), and 60.7 ± 7.8 pg/ml ($n = 9$), respectively. These values were not significantly different from those in the control rabbits at any of the time points (16 h: 73.7 ± 6.5 , $n = 7$; 7 d: 72.6 ± 5.3 , $n = 5$; 14 d: 64.9 ± 7.0 pg/ml, $n = 10$). Thus, UNX had no effects on plasma aldosterone concentration.

Discussion

The present study was designed to determine the cellular mechanisms whereby a reduction of renal mass leads to an increased capacity to reabsorb Na^+ and secrete K^+ in the CCD. We found that UNX stimulated both electrogenic $Na^+ - K^+$ pump activity and K^+ conductance in the basolateral membrane and Na^+ and K^+ conductances in the apical membrane. The CCD from remnant kidneys also exhibited structural hypertrophy. These functional and morphological adaptations occurred independently of changes in plasma aldosterone. This study was the first to demonstrate the electrophysiological properties of the apical and the basolateral membranes of the CD cell from UNX rabbits.

Effects of UNX on electrical properties of the apical membrane. A reduction of renal mass is known to stimulate both Na^+ reabsorption (4) and K^+ secretion (5) in the CCDs from the remnant kidney of the rabbit after nephrectomy. However, whether the changes in membrane conductances contribute to the functional adaptation in the CCD after nephrectomy has not yet been evaluated. In the present study, we demonstrated that UNX led to an increase in G_A .

Na^+ absorption in the rabbit CCD is initiated by passive Na^+ diffusion from the lumen into the cell down its electrochemical gradient via a Na^+ channel and is then actively extruded from the cell into the peritubular space via the $Na^+ - K^+$ exchange pump located in the basolateral membrane (9–11, 15, 16, 21). In the present study, we observed that amiloride-sensitive changes in V_A , fR_A , and G_T were much greater in the UNX group. These observations indicate that amiloride-sensitive Na^+ conductance in the apical membrane is stimulated upon UNX, resulting in an increase in Na^+ / K^+ ATPase activity. Such effects of UNX on the Na^+ conductance are consistent with the reported increase in Na^+ reabsorption in the CCD from the remnant kidneys of the rabbit after nephrectomy.

K^+ is secreted in the rabbit CCD by a two-step process that involves uptake from the blood into the cell via the basolateral membrane $Na^+ - K^+$ pump and passive diffusion down the cell to the lumen through a large apical membrane conductive pathway (9–11, 15, 16, 21, 26). In the present study, we also demonstrated that the changes in V_A upon raising the perfusate K^+ concentration were exaggerated in the CCDs from UNX rabbits. Furthermore, the changes in V_A upon addition of lu-

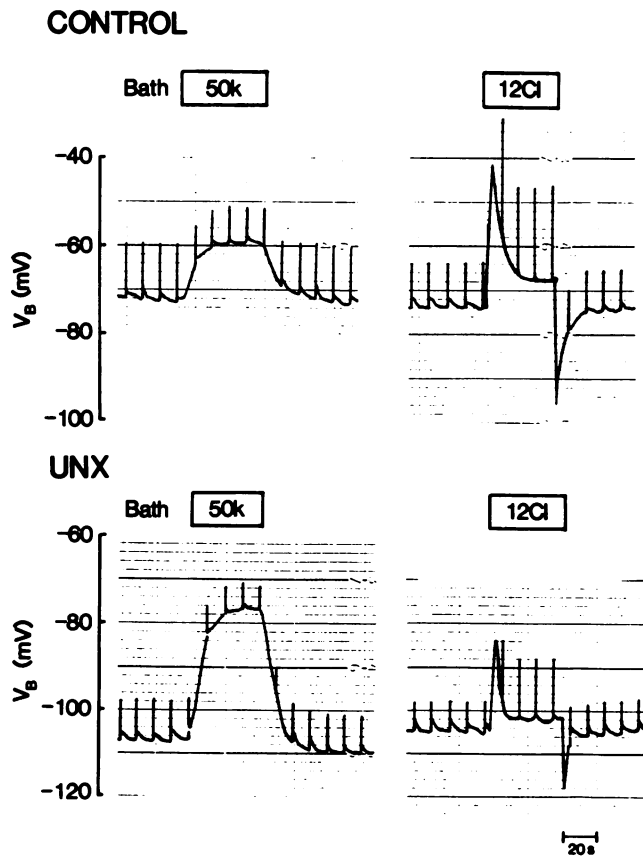


Figure 5. Typical tracings of V_B upon raising bath K^+ concentration and lowering bath Cl^- concentration in the tubules of CONTROL and UNX groups.

Table VIII. Effects of 2 mM Ba²⁺ in the Bath on Barrier Voltages and Conductances

	V _T	V _B	G _T	fR _A
	mV	mV	mS·cm ⁻²	
Control				
Without Ba ²⁺	-6.0±1.6 (25)	-80.0±1.4 (25)	10.3±1.1 (7)	0.50±0.07 (7)
With Ba ²⁺	-5.0±1.9 (25)	-78.4±1.9 (25)	9.5±1.0 (7)	0.46±0.07 (7)
Difference	1.0±0.6	1.6±0.9	-0.8±0.3	-0.04±0.007
P	NS	NS	<0.005	<0.01
UNX				
Without Ba ²⁺	-19.7±1.5 (16)	-101.5±2.9 (16)	11.2±0.8 (10)	0.33±0.03 (10)
With Ba ²⁺	-23.7±1.9 (16)	-106.0±3.1 (16)	8.8±0.6 (10)	0.19±0.03 (10)
Difference	-4.3±0.7*	-4.5±0.6*	-2.4±0.5**	-0.14±0.03**
P	<0.001	<0.001	<0.005	<0.001

Values are mean±SE. Numerals in parentheses indicate number of experiments. * P < 0.001 vs. control; ** P < 0.05. Data were obtained from the same experiments.

minimal Ba²⁺ were also much greater in the UNX group. These findings are consistent with the view that the Ba²⁺-sensitive K⁺ conductance in the apical membrane of the CD cell is also stimulated after UNX. Therefore, the increase in net K⁺ secre-

tion in the CCD from UNX rabbits can be, at least in part, explained by an increase in the apical membrane K⁺ conductance.

Effects of UNX on electrical properties of the basolateral membrane. As shown in Table III, the G_B of the CD cell also appears to be under the influence of UNX. The G_B was observed to increase by a 1.6-fold after UNX. The basolateral membrane conductive pathways of the CD cell in the CCDs from normal rabbits are a dominant Cl⁻ conductive pathway and a small K⁺ conductive pathway (14, 16, 24, 27). Using changes in basolateral membrane electromotive force upon abrupt changes in bath K⁺ and Cl⁻ concentrations, we can estimate the relative conductance of K⁺ and Cl⁻ in the basolateral membrane. Thus, the basolateral membrane of the CD cell in the UNX group is predominantly K⁺ selective. These observations strikingly resemble those seen in the basolateral membrane of the CD cell in the CCD from chronic mineralocorticoid-treated rabbits (24, 26).

In the CCDs from control rabbits, V_B was observed to be near the Nernst equilibrium potential for K⁺ across the basolateral membrane of the CD cell (E_B^K), since addition of Ba²⁺, a blocker of K⁺ channel, to the bath had no effect on V_B. In contrast, after UNX, -V_B was elevated by ~ 20 mV so that a driving force for K⁺ entry into the cell would exist. Under these conditions Ba²⁺ added to the bath could block this inwardly directly passive K⁺ flux and the basolateral membrane should hyperpolarize, as was observed. Similar findings were also reported in the CD cell from chronic DOCA-treated rabbits (24). It can be thus concluded from these considerations that, in the CCDs from UNX rabbits, the basolateral membrane of the CD cell is indeed hyperpolarized above E_B^K, providing a driving force for passive K⁺ uptake across the basolateral membrane. Therefore, increases in both K⁺ conductance and the driving force for K⁺ across the basolateral membrane could result in an increased K⁺ uptake across the basolateral membrane in the CCDs from UNX rabbits.

In the tubules of UNX rabbits of the present study, the basolateral membrane of the CD cell hyperpolarized by ~ 20 mV above the values of the tubules of control rabbits. In addition, the initial peak changes in V_B upon addition of ouabain to the bath were significantly greater in the UNX group, as shown in Fig. 7 and Table IX. From these two observations it is indi-

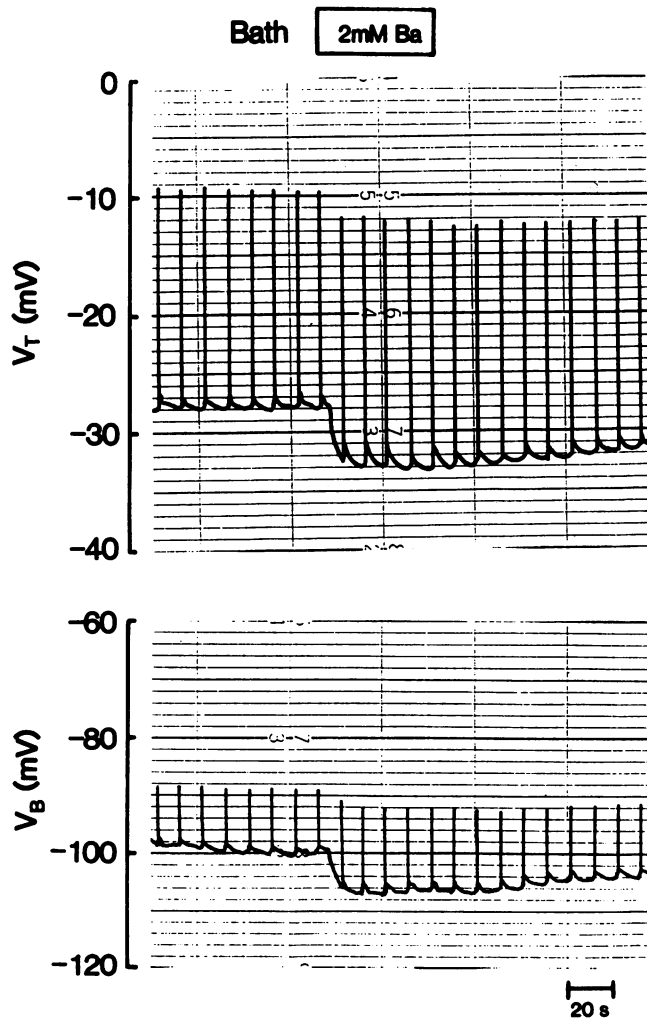


Figure 6. Typical tracings of V_T and V_B upon addition of 2 mM Ba²⁺ to the bath in a tubule from the UNX group.

Table IX. Effects of 10^{-4} M Ouabain in the Bath on Barrier Voltages and Conductances

	V_T mV	V_B mV	G_T $mS \cdot cm^{-2}$	fR_A
Control				
Without ouabain	-12.1 ± 4.4 (9)	-84.4 ± 3.7 (9)	8.6 ± 0.5 (6)	0.44 ± 0.03 (6)
With ouabain	-3.5 ± 3.6 (9)	-74.3 ± 3.9 (9)	8.3 ± 0.6 (6)	0.45 ± 0.03 (6)
Difference	8.5 ± 1.5	10.1 ± 0.9	-0.3 ± 0.2	0.003 ± 0.006
P	<0.001	<0.001	NS	NS
UNX				
Without ouabain	-19.7 ± 2.2 (12)	-101.0 ± 3.6 (12)	10.0 ± 0.6 (5)	0.36 ± 0.03 (5)
With ouabain	-4.8 ± 1.1 (12)	-79.2 ± 3.3 (12)	9.5 ± 0.7 (5)	0.37 ± 0.02 (5)
Difference	$14.8 \pm 1.5^*$	$22.4 \pm 2.0^*$	-0.5 ± 0.4	0.016 ± 0.007
P	<0.001	<0.001	NS	NS

Values are mean \pm SE. Numerals in parentheses indicate number of experiments. * $P < 0.001$ vs. control. Data indicate the initial fast response of each parameter upon addition of ouabain. Data were obtained from the same experiments.

cated that the electrogenic $Na^+ - K^+$ pump activity in the basolateral membrane of the CD cell is stimulated upon UNX, although the maximum pump current was not estimated in the present study. This increase in basolateral pump activity is consistent with biochemical evidence that basolateral Na^+ / K^+ ATPase activity is amplified in the UNX rats (7) and rabbits

(6), and that there is also an amplification of the basolateral membrane area of the CD cell in the initial collecting duct from 75%-nephrectomized rats (8).

Effects of UNX on electrical properties of the tight junction.

In the present study, we demonstrated that UNX caused G_{Tj} to decrease by 32%. Sansom et al. (27), using microelectrode and cable analysis, reported that the paracellular pathway in the rabbit CCD is Cl^- permeable. We speculate thus that UNX would induce a decrease in the conductance of the paracellular Cl^- -conductive pathway. A decrease in G_{Tj} upon UNX may be at least in part responsible for the further increase in the magnitude of the lumen-negative V_T . This would therefore increase the net transepithelial driving force for K^+ secretion.

Mechanisms responsible for the adaptations in the CCD from the remnant kidney after UNX. The mechanisms for the observed adaptations of the apical as well as the basolateral membranes of the CD cell after UNX are not fully understood at the present time. Several factors, including mineralocorticoids (7, 28), Na^+ delivery to the CCD (29–32), and K^+ balance of the animal (5, 33), could be responsible for the increased transport capacity for Na^+ and K^+ in the CCDs from the remnant kidneys.

Mineralocorticoid hormones such as aldosterone are known to increase Na^+ reabsorption and K^+ secretion in the rabbit CCD (11, 16, 20, 21). These effects are accompanied by increasing the activity of Na^+ / K^+ ATPase (18, 19) and the basolateral membrane area of the CD cell (17). This adaptive stimulation in Na^+ reabsorption and K^+ secretion is also attributed to increases in apical membrane Na^+ and K^+ conductances (9, 16, 23) and to an increase in basolateral membrane K^+ conductance (24). Similar changes in membrane conductances of the CD cell after UNX were also observed in the present study. Aldosterone would thus play an important role in regulating the observed adaptation (28). However, our results do not support this hypothesis for several reasons. First, plasma aldosterone levels did not change during the time course of the nephrectomy in this study. Similar results are reported by Fine et al. (5) in 75%-nephrectomized rabbits 3–4 wk after the surgery. Scherzer et al. (6) also arrived at the same conclusion in UNX rabbits 14 d after the surgery. In contrast to our study, Vehaskari and Herndon (28) reported that plasma aldosterone levels were significantly elevated at 16 h and 7 d in

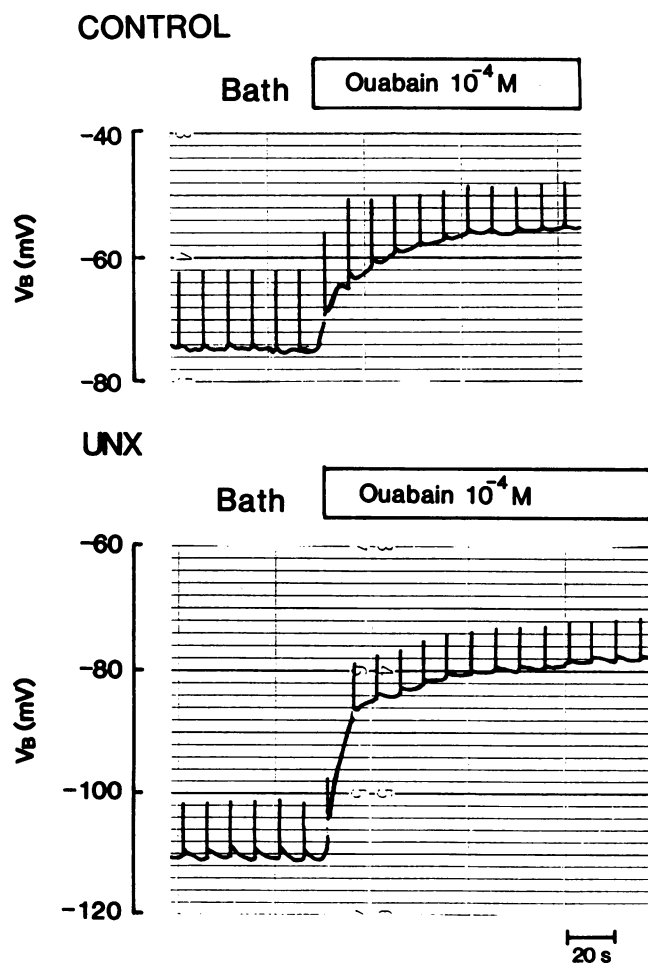


Figure 7. Typical tracings of V_B upon addition of 10^{-4} M ouabain to the bath in the tubules of CONTROL and UNX groups.

75%-nephrectomized rabbits. The reasons for the differences between the two studies are not clear at the present time; the renal function of our remnant kidney animals was, however, within the normal range. On the other hand, the renal function of their animals was moderately impaired (serum creatinine levels: ~ 1.5 – 2.5 mg/dl) due to more extensive reduction of renal mass. According to Hené et al. (34), plasma aldosterone levels are elevated when creatinine clearance is lower than 50% of normal subjects. Accordingly, elevated plasma aldosterone levels in their animals may possibly be due to impaired renal function. Second, a significant and sustained increase in plasma aldosterone levels has not been a consistent finding in situations in which renal mass is reduced to a similar extent as in our experiments. Therefore, we conclude that the observed functional and morphological adaptations in the CCDs from remnant kidneys after UNX are independent of plasma aldosterone.

We also propose that a reduction in renal mass effects to enhance cellular uptake of Na^+ in the apical membrane, and then to stimulate Na^+ absorption. This, in turn, increases the amount of Na^+/K^+ ATPase pump, which is manifested as an increase in the surface area of the basolateral membrane of the CD cell. Several lines of evidence also support this proposal. First, free-flow micropuncture experiments of Diezi et al. (2) showed that UNX increased single-nephron GFR and sodium delivery to the superficial distal tubule, augmenting sodium absorption by this segment. Second, when the delivery of sodium to the distal tubule, including the CCD, is chronically increased by diuretics such as amiloride and furosemide there is an increase in the Na^+/K^+ ATPase activity in this segment, independent of mineralocorticoid (29, 30). Furthermore, Stanton and Kaissling (32) have also shown that a chronic increase in Na^+ delivery to and Na^+ uptake by the distal tubule of the rat in vivo by furosemide administration stimulated Na^+ absorption, independently of a change in aldosterone levels. The amplification of the basolateral membrane area of the CD cell is also associated with an increase in Na^+ delivery to and in cell Na^+ uptake by the CCD (31).

Another factor that would alter the conductances of the apical and the basolateral membranes of the CD cell is dietary K^+ intake. A high K^+ intake has been reported to stimulate Na^+/K^+ ATPase activity in the CCD (35) and to induce the amplification of the basolateral membrane of the CD cell (36), independent of aldosterone. In addition, a high K^+ diet stimulates net Na^+ reabsorption and K^+ secretion in the CCD from adrenalectomized rabbits (37). These changes are also associated with increases in apical Na^+ and K^+ conductances and in basolateral electrogenic Na^+/K^+ pump activity (15). It seems, however, unlikely that changes in K^+ balance or transport would induce the observed adaptation in the CD cell, since plasma K^+ levels were not significantly elevated in the UNX rabbits (Table II). Finally, it is possible that other hormones, such as thyroid hormone and growth hormone, and growth factors may be involved in the mechanisms of the adaptations after nephrectomy.

In summary, we conclude that (a) UNX increases both apical membrane Na^+ and K^+ conductances and basolateral membrane K^+ conductance; (b) the basolateral membrane in the tubules of UNX rabbits is more selective to K^+ ; (c) UNX increases basolateral membrane electrogenic Na^+/K^+ pump activity. This results in a hyperpolarization of the basolateral

membrane, which may increase passive K^+ entry into the cell across the basolateral membrane; and (d) These changes occur independently of plasma aldosterone.

Acknowledgments

We would like to thank Ms. H. Kasakura for expert secretarial assistance in preparing the manuscript.

This work was supported in part by grants from the Japanese Kidney Foundation (Jinkenkyukai) and the Ministry of Education, Science, and Culture of Japan (No. 01570366).

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