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Research Article

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Sodium Transport by Rat Cortical Collecting Tubule

Effects of Vasopressin and Desoxycorticosterone

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Abstract

We have used rat cortical collecting tubules perfused in vitro to study the effects of antidiuretic hormone (ADH) and desoxycorticosterone (DOCA) on the unidirectional fluxes of sodium. We found that in the basal state, lumen-to-bath flux (J_{lb}) and bath-to-lumen flux (J_{bl}) of ^{22}Na were approximately equal, 39.5±3.9 and 41.8±11.0 pmol · min^{-1} · mm^{-1}, respectively, resulting in no net flux. Addition of 100 μU/ml ADH to the bath produced a stable increase in J_{lb} to 58.3±4.7 pmol · min^{-1} · mm^{-1}. Pretreatment of the animal with DOCA for 4 to 7 d (20 mg/kg per d) increased baseline J_{lb} to 81.6±8.7 pmol · min^{-1} · mm^{-1}. Addition of ADH to a tubule from a DOCA-pretreated rat caused an increase in J_{lb} to 144.1±12.0 pmol · min^{-1} · mm^{-1}. Neither hormone had an effect on J_{bl}. Thus ADH produced a greater absolute and fractional increase in J_{lb} when the animal was pretreated with DOCA, and the ADH-induced increase over baseline was greater than the DOCA-induced increase. Both the ADH- and DOCA-induced stimulation of J_{lb} were completely abolished by 10^{-5} M luminal amiloride, suggesting that the route of sodium transport stimulated by both hormones involves apical sodium channels. However, ADH and DOCA have very different time courses of action; ADH acted within minutes, while aldosterone and DOCA are known to require 90–180 min. The facilitating action of ADH on DOCA-induced stimulation of sodium transport may be important for maximal sodium reabsorption and for the ability to achieve a maximally concentrated urine.

Introduction

Arginine vasopressin (antidiuretic hormone [ADH])[1] causes an increase in water permeability and transepithelial voltage (V_e) in the toad bladder (1) and in the isolated perfused cortical collecting tubule (CCT) of the rabbit (2). These effects are transient, for reasons that may involve the local production of prostaglandins induced by ADH (3, 4). Using the isolated perfused CCT of the rat, we have recently shown that, in marked contrast to the situation in the rabbit, the effects of ADH on water permeability and V_e are stable for at least 3 h (5). This stability of the voltage response to ADH was recently confirmed by Tomita et al. (6).

Another hormone with important actions in the mammalian CCT is aldosterone (7). Pretreatment of rabbits with desoxycorticosterone acetate (DOCA), a long-acting synthetic aldosterone, leads to an increase in the V_e, sodium absorption, and potassium secretion. The magnitude of this response increases with duration of the DOCA pretreatment, reaching a maximum after ~2–3 wk of DOCA administration (8).

Studies in the toad bladder (9) and in the frog skin (10) have suggested that ADH rapidly activates preexisting intramembranous sodium channels. The effects of aldosterone appear to be more complex. Aldosterone, like ADH, is able to increase the apical sodium permeability in toad (11) and rabbit urinary bladder (12), but only after a lag time of 45–60 min (13). Aldosterone has additional effects that become manifest only after several hours or days, including increased availability of energy for the sodium pump (14), an increase in the number of pump units (15), and possibly increased synthesis of new sodium channels (12).

Tomita et al. (6) have shown that the rat CCT responds to the chronic administration of DOCA with an increase in net baseline sodium absorption and potassium secretion, and that subsequent in vitro application of ADH produces a stable increase in net sodium absorption and potassium secretion. We have examined the effects of ADH and DOCA on sodium transport in the rat CCT in more detail by measuring V_e and unidirectional transport rates of ^{22}Na, and examining the effect of the apical sodium channel blocker, amiloride. We have found that ADH causes a rapid increase in sodium reabsorption that remains stable with time and that is rapidly reversible upon removal of the hormone. Pretreatment of the animal with DOCA leads to an increase in the baseline lumen-to-bath sodium flux without change in bath-to-lumen flux. The addition of ADH to a DOCA-pretreated tubule causes a much greater increase in sodium absorption than in a nonpretreated tubule. Both the ADH- and DOCA-induced increases in sodium flux are abolished by amiloride. The ability of DOCA to prime the epithelium for an augmented ADH-response may be important for maximal sodium absorption and urinary concentrating ability.

Methods

The animals used in these studies were male Sprague-Dawley rats, weighing 70–100 g, obtained from a barrier-maintained, pathogen-free colony (strain Tac/N [SD] fBR) at Taconic Farms (Germantown, NY). The animals were shipped and housed in special filter-topped containers and kept apart from other animals in barrier-equipped cages with sterilized

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1. *Abbreviations used in this paper:* ADH, antidiuretic hormone; CCT, cortical collecting tubule; DOCA, desoxycorticosterone acetate; J_{bl}, unidirectional bath-to-lumen flux of sodium; J_{lb}, unidirectional lumen-to-bath flux of sodium; V_e, transepithelial voltage.

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food and water ad lib. For an experiment, the animals were killed by decapitation. Both kidneys were rapidly removed; the capsule was stripped off and 4–5 coronal slices made from each kidney. Cortical collecting tubules were dissected at room temperature from the corticomedullary rays. The length of the segments ranged from 0.4 to 1.2 mm. The tubules were mounted and perfused as previously described by us (5, 16) and others (2, 17). We made one modification from the previous perfusion set-up. During measurement of lumen-to-bath fluxes, bathing solution was continuously infused into the Lucite perfusion chamber at a rate of 0.3 ml/min. A small L-shaped steel tube was placed at the top of the bathing fluid level and connected with polyethylene tubing to a scintillation vial that was kept under vacuum. Bathing solution was continuously aspirated into the vial at precisely the rate at which bath was perfused into the chamber, thus keeping the bath volume constant (0.8–1.2 ml). The scintillation vial was exchanged every 10 min and counted by standard double-label techniques with correction for quenching. For any change of bath composition, the volume of the bath was exchanged at 3 ml/min, resulting in a new steady state composition of the bath within 2 to 3 min.

All experiments were carried out at 38°C. Perfusion rates were kept constant during an experiment, but varied from 10 to 21 nl/min between experiments. Samples were collected at 5–10-min intervals in a precalibrated constant-bore collection pipette. The bathing solution contained (in millimolars): NaCl, 124, $NaHCO_3$, 25, Na phosphates (pH 7.4), 2, KCl, 5, $CaCl_2$, 1.5, $MgCl_2$, 0.5, D-glucose, 8.3, L-alanine, 4, Na-acetate, 5, urea, 6, and 1 g/dl of purified bovine albumin, with an osmolality of 310–320 mosmol/kg H_2O. The solution was equilibrated with 95% O_2/5% CO_2 at 38°C and adjusted to pH 7.4 if necessary. The dissection solution was identical to the bathing solution except that the albumin concentration was raised to 5 g/dl. The perfusion solution was always prepared to be isosmotic with the bathing solution and consisted of (in millimolars): NaCl, 140, KCl, 5, $CaCl_2$, 1.5, $MgCl_2$, 0.5, and urea, 50. The solution was equilibrated with 95% O_2/5% CO_2 at 38°C and the pH adjusted to 6.6. Fine adjustments of the osmolality were made with 1 M NaCl or distilled water.

Measurement of lumen-to-bath flux of sodium. For measurement of unidirectional lumen-to-bath flux of sodium (J_{lb}), 25 μCi/ml ^{22}Na was added to the perfusate, as well as 50 μCi/ml of dialyzed [^{3}H]methoxy-inulin as a volume marker. The perfusion rate (V_o) was calculated from the collection rate (V_l), the isotopic concentration of volume marker (in cpm/nl) in the perfusate (C_o), and in the collectate (C_l) in the standard manner, expressed in nanoliters per minute. The [^{3}H]methoxy-inulin was also used as a marker for any leakage of perfusate into the bathing solution.

J_{lb} was calculated from the rate of appearance of tracer in the bath, by the following equation:

$$J_{lb} = C_b^*/(LtS). \quad (1)$$

C_b^* is the total cpm ^{22}Na in the collected bathing fluid, L the tubule length in millimeters, t time in minutes, and S the specific activity, determined as the quotient of the measured radioactivity (in cpm/nl) and the known concentration of Na^+ (in pmol/nl) of the perfusate. J_{lb} was corrected for the apparent bulk leak rate assessed by ^3H appearance in the bathing solution. Experiments with a bulk leak rate of >1% of the total perfusion rate were discarded.

In each experiment, J_{lb} was also calculated from the differences in concentration of ^{22}Na between perfusate and collectate, and excellent agreement was found between the results obtained by the two calculations. All results reported here are calculated from Eq. 1.

Measurement of bath-to-lumen flux of sodium. For measurement of unidirectional bath-to-lumen flux of sodium (J_{bl}), 50–100 μCi ^{22}Na and 50 μCi [^{3}H]methoxy-inulin were added to a constant bath volume of 250 μl. The bathing solution was covered with a layer of dimethylpolysiloxane (200 Fluid, Dow Corning Corp., Midland, MI) stained with Oil Red O, and superfused with a mixture of 20% CO_2/80% O_2 at a rate sufficient to maintain pH at 7.4. We have verified that this method maintains constancy of pH while preventing significant evaporation of the bath. J_{bl} was calculated from the rate of appearance of isotope in the collected fluid, as:

$$J_{bl} = \frac{[C_l^* V_l (C_b/C_b^*)]}{(L)}, \quad (2)$$

where C_b and C_b^* are the chemical (in mM) and isotopic (in cpm/nl) concentrations in the bathing solution. Eq. 2 assumes that the luminal isotope concentration remains considerably less than that in the bathing solution, so that the backflux from the lumen to the bath is negligible. In our experiments, the cpm/nl of the collectate was consistently below 2% of the cpm/nl in the bath. The leak rate was determined from the amount of [^{3}H]methoxy-inulin appearing in the collectate, and the calculated J_{bl} was corrected for leak. Any experiment with a leak from bath-to-lumen equivalent to >1% of the total volume of the collectate was discarded.

Measurement of V_e. In most experiments, V_e was continuously recorded between calomel electrodes connected via standard bridges of 0.9% NaCl in 4% agar that were inserted in the perfusion pipette and bathing solution. The voltage difference was measured using a high impedance electrometer. In the reported voltages, no correction was made for the junction potentials because the perfusion and bathing solutions used were the same in all experiments, so that the junction potentials were constant. Also, we were interested primarily in changes in voltage rather than absolute voltages. We computed the average liquid junction potential to be 1.9 mV using the Henderson-Planck equation (18) and the measured concentrations of Na^+, K^+, Cl^-, and HCO_3^- in these experiments. The reported voltages may therefore be corrected by adding +1.9 mV to obtain the actual V_e.

Administration of hormones. ADH was added to the bath at 100 μU/ml, a concentration shown by us to be supramaximal for this preparation (5). DOCA was administered intramuscularly at a dose of 20 mg/kg daily for 4–7 d before the experiment. In other experiments, aldosterone was added to the bath at a concentration of 10^{-6} M.

Sources of biochemicals. Synthetic arginine vasopressin was obtained as an aqueous solution from Parke-Davis Corp. (Morris Plains, NJ). (+)Aldosterone was from Calbiochem-Behring Corp. (San Diego, CA). All radiolabeled compounds were purchased from New England Nuclear (Boston, MA). Amiloride was a gift from Merck, Sharp & Dohme (West Point, PA); it was obtained as a powder and stored as an aqueous 10^{-3}-M stock solution at 3°–5°C.

Statistical analysis. 4–6 collections per experimental period were used to obtain a mean value for that period. Mean values for individual tubules were then used to compute a mean value±SEM for the number of tubules indicated. Means between experimental periods were compared by a paired t test. Comparisons between groups were made by Duncan's new multiple range test (19). Where the t test was used, the P levels are indicated, while Duncan's test was performed for a significance level of $P < 0.05$ only.

Results

The effect of ADH on baseline J_{lb} was determined in 13 tubules from untreated rats. V_e was recorded simultaneously in all of these experiments. After a control period of 45–60 min, 100 μU/ml ADH was added to the bath, and perfusion continued for ~60 min. The results of these experiments are shown in Fig. 1 and Table I. The effect of ADH on voltage was apparent within 2 to 3 min. Although the time intervals between fluid collections do not permit such a time resolution for flux measurements, an increase in J_{lb} was always seen in the first collection after the addition of the hormone to the bath.

In four of these tubules, we measured J_{lb} and V_e after rapid removal of ADH from the bath. J_{lb} was observed to fall dra-

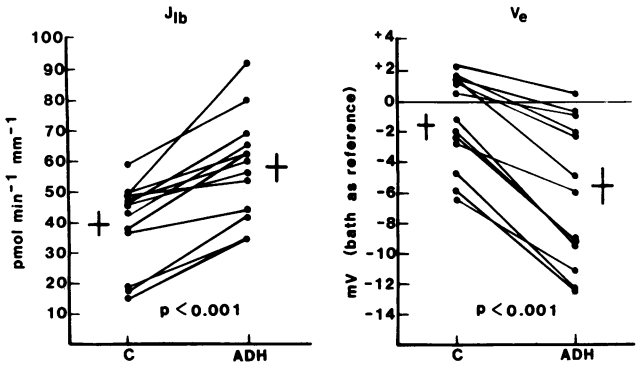

Figure 1. Effect of ADH on J_{lb} and V_e. After a control period (C), 100 μU/ml ADH was added to the bath (ADH). n = 13; bars denote mean±SEM.

matically in the first collection after ADH withdrawal, and V_e returned to control levels within 2 to 3 min. These data are shown in Fig. 2. In these four tubules, J_{lb} was lower after removal of ADH than before the addition of the hormone, but V_e remained unchanged.

In four separate experiments, ADH was maintained in the bath for 180 min. J_{lb} was 51±9.3 pmol·min^{-1}·mm^{-1} during the first 90 min, and 50.9±7 pmol·min^{-1}·mm^{-1} during the following 90 min, with no significant correlation between flux and elapsed time (n = 4). V_e was −4.9±1.3 mV during the first 90-min period, and −5.9±1.5 mV during the following 90 min (NS). The prolonged and stable response of J_{lb} to ADH is consistent with our earlier findings of a stable voltage response (5).

The response of J_{bl} to ADH was studied in the next series of experiments (see Table I). ADH was without effect on J_{bl}. V_e was measured in two of these seven tubules, and was comparable to the voltages seen in the J_{lb} experiments. J_{bl} showed a greater variability from tubule to tubule than did J_{lb}, but the mean J_{bl} in the absence of ADH was very close to the mean value for J_{lb}, indicating no net absorption in the unstimulated state. ADH did not significantly increase net sodium absorption calculated from unpaired experiments due to the inherent variability of the data.

In the next set of experiments, we examined the effect of aldosterone in vitro on J_{lb} and V_e. We added 10^{-6} M aldosterone to the bath and perfused the tubules 110–188 min (n = 4). The time courses of J_{lb} and V_e for these experiments are shown in Fig. 3, *A* and *B*. We were unable to detect any change in J_{lb} or V_e with aldosterone during this time span. The one tubule with the smallest initial J_{lb} did show a monotonic increase after 120 min in the presence of aldosterone, but there was no change in V_e during that time.

We then examined sodium fluxes in tubules from animals that had been pretreated chronically with DOCA. In the first group of experiments (10 tubules) we measured J_{lb} during a control period and after addition of ADH. We found a marked stimulation by ADH. These results are in Fig. 4 and Table I. The bath-to-lumen fluxes, J_{bl}, were measured in four tubules, and were not significantly different from control animals. ADH, in the DOCA-treated tubules, did not appear to affect J_{bl}. V_e was measured in one of these four tubules, and was −3.1 during control and −9.5 mV with ADH.

A comparison of the results between control and DOCA-pretreated rats is shown in Table I. DOCA treatment significantly increased baseline J_{lb} and V_e, without affecting J_{bl}. The effect of ADH on J_{lb} was significantly greater in DOCA-pretreated tubules. The same is true for the hyperpolarization of the V_e caused by

Table I. Effects of ADH and DOCA on V_e, J_{lb}, J_{bl}, Net Flux of Sodium (J_{net}), and V_e

	Baseline	ADH	Change with ADH
V_e			
Normal rats (13)	−1.3±0.8	−6.1±1.3	−4.8±0.7
DOCA rats (10)	−8.3±1.5	−26.3±4.3	−18.0±3.1
Comparison, normal vs. DOCA rats	*	*	*
J_{lb}			
Normal rats (13)	39.5±3.9	58.3±4.7	+18.9±2.7
DOCA rats (10)	81.6±8.7	144.1±12.0	+62.6±7.4
Comparison, normal vs. DOCA rats	*	*	*
J_{bl}			
Normal rats (7)	41.8±11.0	39.4±11.3	−2.4±1.8
DOCA rats (4)	47.1±11.2	33.7±4.7	−13.4±9.2
Comparison, normal vs. DOCA rats	NS	NS	NS
J_{net}			
Normal rats	−2.3±11.7	18.9±12.2	+21.2±16.9
DOCA rats	34.5±14.2	110.4±12.9	+75.9±19.2

Voltages are from J_{lb} experiments. Units are pmol·min^{-1}·mm^{-1} (J_{lb} and J_{bl}), and millivolts (V_e). Values are mean±SEM; numbers of tubules are in parentheses. * Indicates significance by Duncan's new multiple range test.

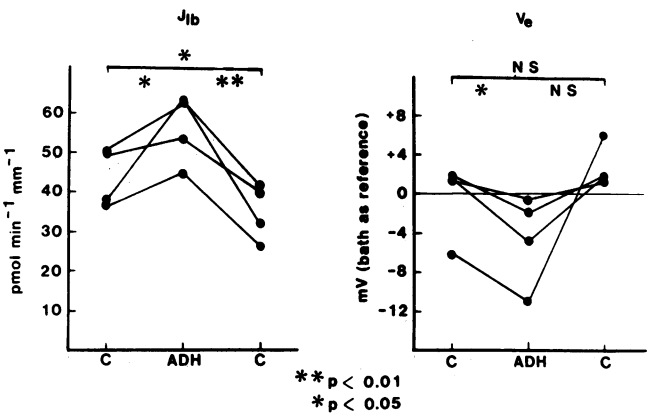

Figure 2. Reversibility of the effect of ADH. After an initial control period (C), 100 μU/ml ADH was added to the bath. At the beginning of the third period (C), ADH was rapidly flushed out from the bath.

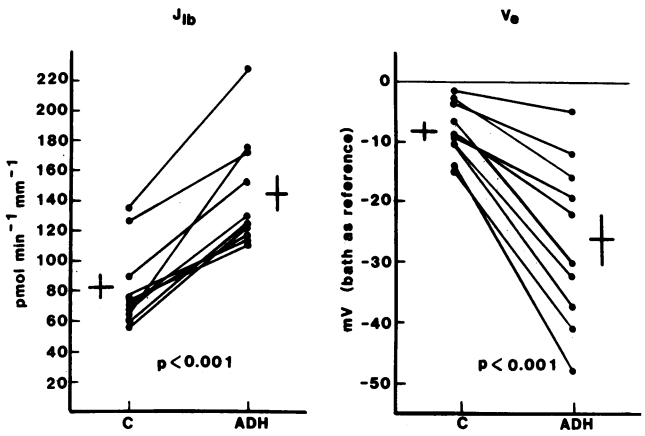

Figure 4. Effect of ADH in DOCA-pretreated rats. The animals were given DOCA 20 mg/kg per d for at least 4 d before the experiment. After an initial control period (C), 100 μU/ml ADH was added to the bath. J_{lb} is shown at left, V_e at right. *n*, 10 tubules.

ADH. The difference in net sodium absorption caused by ADH is even more impressive if one considers that baseline net sodium absorption is close to zero in untreated rats. The fractional increase in J_{lb} (increase in J_{lb} divided by baseline J_{lb}) caused by ADH is 0.48 in tubules from normal rats and 0.77 after DOCA pretreatment. It seems clear that pretreatment with DOCA in some way conditions the epithelium for a maximal ADH response.

Because both hormones act, at least in part, on the amiloride-sensitive apical sodium channel (12), we examined the effect of the addition of 10^{-5} M amiloride to the lumen on J_{lb} and V_e.

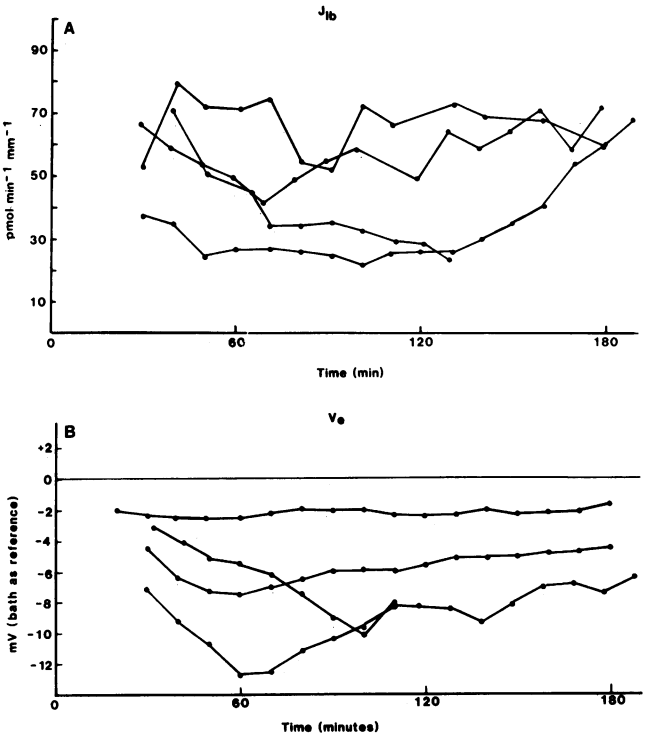

Figure 3. (*A*) Absence of an effect of aldosterone in vitro on J_{lb}. During the entire perfusion period, 10^{-6} M aldosterone was present in the bath. (*B*) Absence of an effect of aldosterone in vitro on V_e. Same tubules as in *A*.

We performed two sets of experiments: the first set consisted of the addition of ADH to the bath (after an initial control period), followed by the addition of amiloride in the continued presence of ADH. These results are shown in Fig. 5 *A* (left panel). In this group, amiloride caused a significant decrease in the ADH-stimulated J_{lb}, returning it to a value not significantly different from control despite the continued presence of ADH. The values were (in pmol · min^{-1} · mm^{-1}): C: 31.0±9.0; ADH: 52.2±9.4; and ADH + amiloride: 27.8±5.8. In a second set of experiments, the sequence of the addition of ADH and amiloride was reversed. This group is shown on the right panel of Fig. 5 *A*. The values were for C: 46.6±10.4; amiloride: 29.5±7.0; and amiloride + ADH: 32.6±6.3 pmol · min^{-1} · mm^{-1}. Thus, in the presence of amiloride, ADH had no detectable effect, and J_{lb} during the third period (ADH + amiloride) was not significantly different from control (C), even when the data of the third period of both sets of experiments were pooled (see Table II). V_e was hyperpolarized by ADH, and that increase also was abolished by amiloride, as shown in Fig. 5 *B*. V_e in the presence of amiloride was not statistically different from control, whether the two groups were analyzed separately or whether the data were pooled (see Table II).

The first set of experiments (C − ADH − ADH + amiloride) was repeated in DOCA-pretreated rats (n = 4), shown in Fig. 6 and Table II. Amiloride suppressed all DOCA- and ADH-induced increase in J_{lb}. J_{lb} and V_e in the presence of ADH plus amiloride were the same in normal and in DOCA rats. The effect of amiloride on V_e was immediately reversible upon removal of amiloride from the lumen. The reversibility of the amiloride effect on J_{lb} was not tested.

As shown in Table II, in control tubules, J_{lb} and V_e in the presence of amiloride (with or without ADH) were suppressed to values not statistically different from the baseline values. In DOCA-pretreated tubules, J_{lb} and V_e were also suppressed to the same baseline value of normal tubules. We did not test the effect of amiloride in the absence of ADH in DOCA-treated rats, and cannot exclude that DOCA has an amiloride-independent effect on J_{lb}. Recent work by Sansom and O'Neil (20) in the rabbit CCT showed a decrease in paracellular conductance after chronic treatment with DOCA. Such a decrease in shunt con-

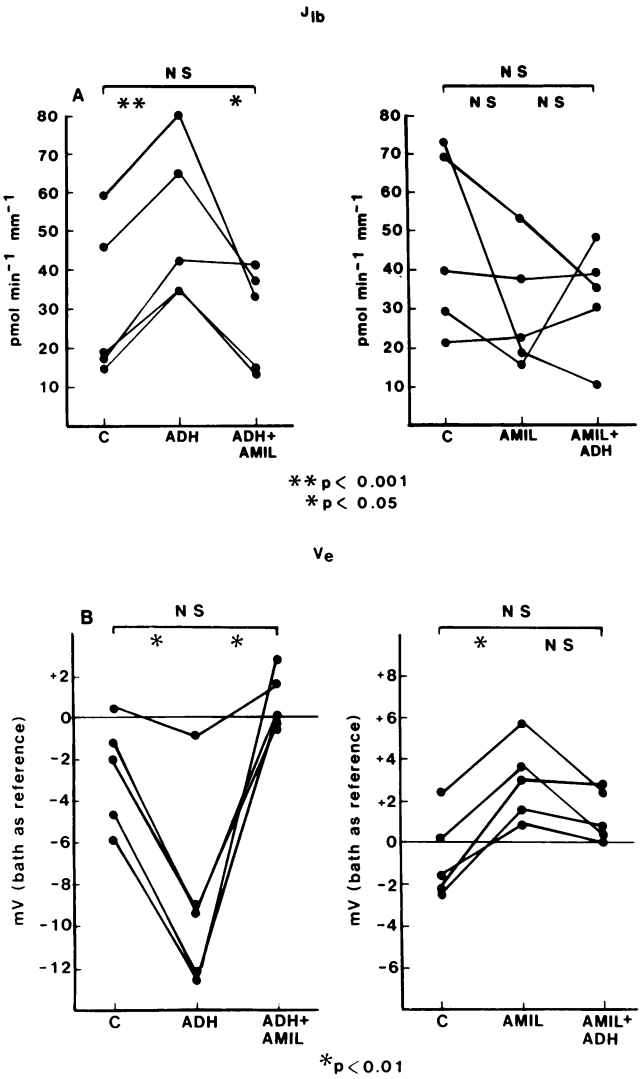

Figure 5. (*A*) Effect of amiloride (AMIL) on ADH-stimulated sodium absorption (J_{lb}). After an initial control period (C), 100 μU/ml ADH was added to the bath (ADH). During the third period, 10^{-5} M amiloride was added to the lumen (ADH + AMIL), shown on the *left* panel. In the second set of tubules, the sequence was reversed (*right* panel). (*B*) Effect of amiloride on ADH-stimulated V_e. The experimental periods are labeled as in Fig. 5 *A*.

ductance leads to an increase in V_e and a secondary increase in J_{lb}. At present, we are unable to assess the role of changes in paracellular conductance on J_{lb}.

Discussion

The data presented above show that in the unstimulated state, the rat CCT exhibits no net sodium absorption. ADH caused a rapid and sustained increase in J_{lb} without affecting J_{bl}. The stimulation of J_{lb} was reversible, and was accompanied by hyperpolarization of the lumen-negative V_e. CCT dissected from animals that had been pretreated with DOCA exhibited net sodium absorption, which was markedly increased by in vitro application of ADH. Neither ADH nor DOCA significantly affected J_{bl}. Both the DOCA- and ADH-induced increments in Na^+ absorption and V_e were completely inhibited by luminal amiloride.

Interactions between adrenal corticosteroids and ADH in modulating Na^+ transport have previously been examined only in the toad bladder (21–23). Studies in the rabbit CCT showed a transient hyperpolarization of the V_e with ADH, accompanied by an increase in J_{lb} with no change in J_{bl} (24). However, the increase in J_{lb} peaked within 10 min and then J_{lb} fell to below control levels over a 30-min period (25). Even in the toad bladder the response to ADH is transient and the increased Na^+ flux begins to fall off within 15 to 60 min after hormone addition (21). In contrast, in the rat CCT, ADH produces a stable increase in both water permeability and V_e for a period of at least 3 h (5), and, as shown in the present studies, a parallel increase in the lumen-to-bath Na^+ flux. This stability of the response may be due to a lack of inhibition by prostaglandin E_2 observed in the rat CCT (25), in contrast to findings in the rabbit CCT (26, 27). Thus, the rat CCT would appear to be an ideal tissue in which to study the mechanisms involved in the stimulation of Na^+ transport by ADH.

The response of the rat CCT to ADH is quite rapid. Within the first 2–3 min after ADH addition, the V_e began to hyperpolarize, and there was an increase in J_{lb} observed within the first 10-min flux period. In contrast, J_{bl} remained unaffected by either ADH or DOCA. Furthermore, in tubules from either control or DOCA-treated animals, amiloride reduced J_{lb} to values not significantly different from J_{bl} even in the presence of ADH (see Table II). These observations suggest that J_{bl} represents a paracellular and/or a basal transcellular Na^+ movement that is insensitive to amiloride. If this were the case, one might expect J_{bl} to vary with V_e; however, the predicted changes in J_{bl} for the variations in voltage observed are smaller than can be resolved by these experiments. Assuming that bath-to-lumen fluxes do occur by a passive permeation pathway, these data together with the measured V_e (corrected for liquid junction potentials) can be used to calculate an apparent sodium permeability using the Goldman-Hodgkin-Katz approximation (28). This permeability, normalized for the apparent luminal surface area ranged from 0.070 to 0.085 μm/s for the various experimental protocols, but there was no significant difference produced by treatment with ADH or DOCA. Although this sodium permeability is about tenfold higher than that measured in the rabbit CCT (29), it is still sufficiently low to allow the development of a steep lumen to plasma Na^+ concentration gradient in this segment.

In our experiments, pretreatment with DOCA for 4–8 d markedly increased J_{lb} and V_e, much like the findings in the rabbit (8, 9). Due to the large doses of DOCA given, we cannot distinguish whether these effects are due to mineralocorticoid or glucocorticoid actions or both. In contrast to the in vivo treatment with DOCA, aldosterone administered acutely to the bathing solution for up to 3 h increased J_{lb} in only one of four tubules, and then only after 120 min, and there was no change in V_e (see Fig. 3). These results contrast with those of Gross and Kokko (30), who observed an increase in V_e in the rabbit CCT after only 10–20 min in the presence of aldosterone in vitro. The same laboratory also reported an increase in Na^+ transport within 3 h of in vitro aldosterone treatment in the rabbit CCT (31). However, this increase was not accompanied by any change in voltage, and may involve an electroneutral transport process. Our studies do not confirm these findings, but cannot rule out a small electroneutral stimulation of J_{lb} by aldosterone. In the toad bladder, Na^+ absorption begins to increase ~45 min after aldosterone addition to the bathing solution (13). Nevertheless,

Table II. Effect of Amiloride on J_{lb} and V_e in Normal and DOCA-pretreated Rats

	Baseline	ADH alone	Amiloride alone	ADH + Amiloride
J_{lb}				
Normal rats	38.8±7.0	52.2±21.0	29.5±7.0	30.2±4.1
	(10)	(5)	(5)	(10)
P vs. baseline	—	NS	NS	NS
DOCA rats	85.8±16.7	143.9±28.4	—	47.8±15.8
	(4)	(4)		(4)
P vs. baseline	—	<0.05	—	<0.02
P, normal vs. DOCA rats	*	*		NS
V_e				
Normal rats	−1.7±0.8	−8.9±2.1	+3.0±0.9	+1.0±0.4
	(10)	(5)	(5)	(10)
P vs. baseline	—	*	NS	NS
DOCA rats	−4.2±1.6	−13.7±3.6	—	+0.6±0.5
	(4)	(4)		(4)
P vs. baseline	—	<0.05	—	NS
P, normal vs. DOCA rats	NS	*		NS

Units are pmol · min^{-1} · mm^{-1} (top half [J_{lb}] of table) and millivolts (bottom half [V_e] of table). Values are mean±SEM; numbers of tubules are in parentheses. Comparisons between experimental periods were done by paired *t* test (*P* levels are shown), and between groups of pooled data and between groups of normal and DOCA-treated animals by Duncan's new multiple range test, where * indicates significance.

the important point to note is the much more rapid action of ADH in comparison to aldosterone or DOCA in both our and other studies.

In the absence of ADH or DOCA treatment, we observed no net Na^+ flux, i.e., there was no statistically significant difference between J_{lb} and J_{bl} (see Table I). Addition of ADH to the bathing solution significantly increased J_{lb} with no effect on J_{bl}; however, the difference between J_{lb} and J_{bl} was still not statistically significant. This absence of statistical significance appears to be attributable to the variability in J_{bl}, and a true stimulation of net sodium absorption by ADH probably does occur. The observed hyperpolarization of the voltage is consistent with that finding.

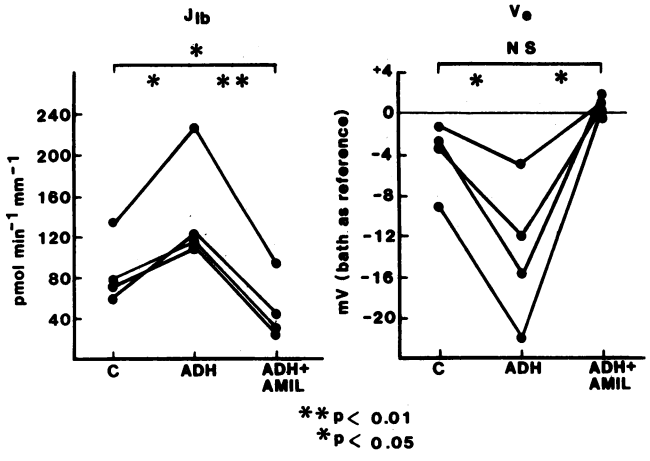

Figure 6. Effect of amiloride (AMIL) on ADH-stimulated sodium absorption (J_{lb}) and V_e in tubules from DOCA-pretreated rats. The experimental periods are labeled as in Fig. 5 *A*.

In CCT from DOCA-pretreated rats, there was significant net Na^+ absorption, which was further stimulated by ADH. This change in net absorption caused by ADH was about twice that produced by DOCA alone (see Table I). Our calculated net fluxes are in reasonable agreement with those of Tomita et al. (6), who measured net fluxes in the rat CCT using the helium-glow photometer. These authors found no net Na^+ flux in the absence of ADH or DOCA, and a net flux of 58.0±11.3 pmol · min^{-1} · mm^{-1} after treatment with both hormones. With ADH and DOCA present, we calculated a net sodium absorption of 110.4±12.9 pmol · min^{-1} · mm^{-1}. If one assumes a length of 1.5 mm for the rat CCT (32), the amount of sodium reabsorbed by this segment will vary from zero to up to 2.5% of the filtered load in a maximally stimulated animal. The CCT may therefore play a significant role in overall sodium balance. Enhanced solute removal by the CCT may also be an important contributor to maximal urinary concentrating ability (33), and the increased Na^+ absorption may also be linked to enhanced K^+ secretion during antidiuresis (34, 35).

Of particular interest in these studies is the augmentation of the ADH effect by pretreatment with DOCA. The increment in J_{lb} produced by ADH is three times greater in DOCA-treated tubules than in controls. This effect is even more evident when considering the increment in the net flux produced by ADH after DOCA treatment (see Table I). A similar effect of aldosterone has been reported in the toad bladder. Although Crabbé (21) reported that ADH produced an equal increment in net Na^+ transport (assessed by the short-circuit current) when added to control and aldosterone-treated bladders, it was subsequently demonstrated that if the toad bladders were depleted of endogenous steroid, subsequent aldosterone treatment resulted in a larger absolute increment in Na^+ transport in response to ADH (22, 23). At first these effects were attributed to different sites of

action of the two hormones. It was thought that ADH influenced only the Na^+ permeability of the apical membrane, while aldosterone augmented the operation of the basolateral Na,K-ATPase either directly or through enhanced production of metabolic intermediates (22).

Enhanced apical Na^+ entry produced by ADH was originally demonstrated by increased apical membrane conductance (36) and by increased $^{22}Na^+$ uptake across the apical membrane (37). Subsequently, the use of noise analysis techniques in both the toad bladder (10) and frog skin (11) showed that ADH produced an increase in the number of Na^+-selective channels in the apical membrane with no change in their unit conductance. However, the same methods have been used to show that aldosterone also increases the number of apical Na^+ channels (38). In fact, the earliest response of the toad bladder to aldosterone, after a 45-min latent period, is an increase in apical membrane conductance followed only after 6 h by an increase in the synthesis of α- and β-subunits of the Na,K-ATPase (15, 39).

The above results indicate that both ADH and aldosterone increase the number of apical membrane Na^+ channels, although aldosterone has additional modes of augmenting Na^+ transport in the toad bladder. In the present studies, the fact that amiloride completely inhibits both the ADH- and the DOCA-dependent increase in J_{lb} suggests that both hormone effects involve transport exclusively through amiloride-sensitive Na^+ channels. The important question is why there is a greater increase in net Na^+ flux with ADH after DOCA treatment than with DOCA alone. Apparently maximal corticoid stimulation is not sufficient to induce maximal Na^+ transport unless ADH is also present.

One possible explanation for the interaction between the two hormones is that aldosterone or DOCA might increase the efficiency of intracellular cyclic AMP production by ADH. It has been shown that adrenalectomy causes an impairment of coupling between the ADH receptor and adenylate cyclase activity in rat medulla (40). In the toad bladder, aldosterone enhanced the response of Na^+ transport to cyclic AMP, which indicates that the steroid does more than enhance hormone-receptor coupling (41). In the same tissue it was found that aldosterone also decreased the activity of cyclic nucleotide phosphodiesterase, which could produce higher levels of cyclic AMP in response to ADH (41). Alternatively, or in addition, maximal apical membrane Na^+ permeability might only be achieved in the presence of ADH. DOCA is known to increase basolateral membrane surface area (42) and Na,K-ATPase activity in the rabbit CCT (43). This effect would provide a greater potential for Na^+ transport, which could only be fully expressed with a maximal increase in Na^+ permeability of the apical membrane, requiring the presence of ADH.

From our data and those obtained in other epithelia, it seems likely that both hormones act on the apical Na^+ channel, but via different mechanisms. One possibility is that ADH transfers Na^+ channels from an inaccessible, probably subapical pool to the membrane (44), but does not alter the proportion of inactive to active channels (45). DOCA, on the other hand, may convert inactive channels into active ones, but not change the proportion of inaccessible to accessible Na^+ channels (46). Thus, ADH in the absence of DOCA would cause insertion of a mixture of active and inactive channels into the membrane, and DOCA in the absence of ADH would not maximally elevate Na^+ permeability because the majority of channels would reside in the inaccessible pool. However, in the DOCA-treated tissue, ADH would produce a maximal increase in Na^+ entry by the insertion of mostly activated Na^+ channels.

Although our data do not allow the determination of the cellular location of the observed synergism, the questions raised by these observations should lead to further investigation into the mechanisms of the hormonal regulation of Na^+ transport. Of equal importance will be to determine the relative importance of aldosterone and ADH in increasing Na^+ excretion versus the enhancement of urinary concentrating ability.

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