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Aldosterone regulation of active sodium chloride transport in the lizard colon *(Gallotia galloti)**

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Summary. Bioelectrical parameters and unidirectional sodium and chloride fluxes were measured under voltage-clamp conditions in groups of lizards submitted to single or chronic aldosterone treatment. Both acute (AT) and chronic (CT) treatment induced significant increases in the short-circuit current (I_{sc}), as well as in the mucosa-to-serosa (J^{Na}_{m-s}) and net sodium flux (J^{Na}_{net}). In AT tissues, aldosterone did not change net chloride flux (J^{Cl}_{net}) but did so in CT tissues. Amiloride reduced the aldosterone-increased I_{sc} in AT and CT tissues, inhibited J^{Na}_{net} in AT tissues and abolished it in CT colons. J^{Cl}_{net} was also reduced by the diuretic in the group of AT colons, whereas no changes were observed in the CT tissues. Addition of luminal DIDS reduced Na^+ absorption and totally inhibited Cl^- absorption in the AT tissues, but did not change I_{sc}. However, in CT tissues neither Na^+ nor Cl^- transport were affected by DIDS. A good relationship between I_{sc} and J^{Na}_{m-s} was apparent after DIDS treatment in AT tissues. In this group, simultaneous addition of DIDS and amiloride totally abolished J^{Na}_{net} and reduced I_{sc} to untreated control values. Addition of serosal ouabain abolished I_{sc} and Na^+ absorption in AT and CT colons, but Cl^- absorption was only altered in AT tissues. These results support the hypothesis that aldosterone induces an electrogenic, amiloride-sensitive sodium absorption, and in a dose-dependent fashion suppresses electroneutral NaCl absorption in the lizard colon.

Key words: Aldosterone – Electroneutral sodium chloride transport – Electrogenic sodium absorption – lizard, *Gallotia galloti*

Abbreviations: AT, acutely treated; CT, chronically treated animals; DIDS, 4-4′-diisothiocyanatostilbene-2-2′-disulfonic acid; DMSO, dimethylsulphoxide; G_t tissue conductance; I_{sc}, short circuit current; PD, transepithelial potential difference; SITS, 4-acetamido-4′-isothiocyanatostilbene-2-2′-disulfonic acid; UC, untreated controls;

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Introduction

Aldosterone has been shown to stimulate Na^+ absorption across a variety of epithelia, such as the renal collecting tubule (Fanestil and Park 1981), urinary bladder (Eaton 1981), colon (Frizzell and Schultz 1978; Clauss et al. 1984), skin (Crabbe 1980) and coprodeum (Thomas and Skadhauge 1979; Thomas et al. 1980). Although in these epithelia the primary action of aldosterone is the stimulation (or induction) of electrogenic Na^+ absorption, aldosterone has also been shown to modify electroneutral NaCl absorption. In the rat distal colon, aldosterone infused for 24 h increases electroneutral NaCl absorption, while aldosterone infusioned for 7–10 days result in its inhibition (Foster et al. 1983; Halevy et al. 1986). Examination of in vitro ion transport under voltage-clamp conditions has established that Na^+ depletion augments electrogenic Na^+ absorption, induces amiloride sensitivity, and inhibits electroneutral NaCl absorption in the rat distal colon (Foster et al. 1983; Halevy et al. 1986). Since Na^+ depletion is associated with secondary hyperaldosteronism, it is likely that these changes in NaCl transport result directly from the action of aldosterone on the colonic epithelium. These studies also revealed that the time-course for the induction of changes in the colonic epithelial cell function by aldosterone are not identical, and that both the duration and magnitude of high aldosterone plasma levels are determining factors of the observed alterations of NaCl transport (Halevy et al. 1986).

Aldosterone effects on "leaky" epithelia have been relatively poorly studied and, until quite recently, it was thought that aldosterone acted solely on "tight" epithelia to enhance Na^+ transport. However, there is indirect evidence suggesting that "leaky" epithelia may be responsive to aldosterone (Will et al. 1985; Grubb and Bentley 1987).

The mechanisms of NaCl transport in the lizard colon have been recently characterized (Badía et al. 1987). In this species the colonic epithelium is considered to be a "leaky" epithelium, in which the predominant pathway for Na^+ absorption is electroneutral and coupled to Cl^-

absorption. The aim of the present work was to examine the effects of aldosterone on electrolyte transport across the lizard colon.

Materials and methods

Collection of animals and tissue incubation. Adult male and female *Gallotia galloti* lizards were transported to the laboratory and acclimatized in a large indoor terrarium for 2–4 days before being used for experiments. Mean body weight of experimental animals was 36.43 ± 2.13 g. Colons were removed after decapitation, opened along their mesenteric border, rinsed free of luminal contents and immersed in iced Ringer solution until the time of mounting. The standard (KRB) solution contained (in mmol · l^{-1}): NaCl 107; KCl 4.5; $NaHCO_3$ 25; Na_2HPO_4 1.8; NaH_2PO_4 0.2; $CaCl_2$ 1.25; $MgSO_4$ 1.0; and D(+)glucose 12. Additionally, solutions were gassed with a mixture of 5% CO_2/95% O_2, resulting in a pH of 7.4 at 30 °C, the temperature at which experiments were performed.

All studies were performed using standard Ussing-type chambers (0.21 cm^2 exposed surface area). After mounting the tissue, 4 ml Ringer solution was added to both mucosal and serosal sides. Solutions were recirculated by gas lift and thermostatically kept at 30 °C in water-jacketed half-chambers during the experiment. Inhibitors were added to respective reservoirs in small volumes from concentrated stock solutions to obtain a final bath concentration of 10^{-4} *M* for amiloride and ouabain and 10^{-3} *M* for DIDS. Each tissue chamber was connected to an automatic computer-controlled voltage-clamp device (AC-microclamp, Aachen, FRG) that allowed continuous measurement of the short circuit current (I_{sc}) and compensation for solution resistance. The transepithelial potential difference (PD), tissue conductance (G_t) and I_{sc} were obtained as reported previously (Díaz and Lorenzo 1990).

Flux measurements. Approximately 5 μCi ^{22}Na or ^{36}Cl was added to either the mucosal or serosal bath after voltage clamping. Preliminary observations indicated that stable flux rates were achieved within 30 min after isotope addition. Thus, flux determinations were initiated after a minimum waiting period of 30 min. Unidirectional mucosa-to-serosa (J^e_{m-s}) and serosa-to-mucosa (J^e_{s-m}) fluxes were calculated from two 200-μl aliquots taken every 20 min during an initial 60-min flux period (pre-inhibitor) and a second 60-min flux period after the addition of diuretics (post-inhibitor) from the unlabelled side. Comparisons of drug effects and their corresponding controls were carried out as pre-inhibitor versus post-inhibitor periods. The activity of radioisotopes in the flux samples was determined by using a β-counter (LKB–1209, Rackbeta). To calculate the unidirectional fluxes, the steady-state rates of radioisotope transfer were divided by the specific activity of the initially labelled side and by the surface area of exposed tissue, according to standard equations from Schultz and Zalusky (1964) with a computer program developed in our laboratory (Díaz and Cozzi 1991). The specific activity for each radioisotope was calculated at the beginning of each period, so that the radioactivity appearing on the unlabelled side could be validated for ionic changes after the addition of inhibitors. The net flux was calculated as the difference between opposing unidirectional fluxes. Net residual flux was calculated by substracting the difference between sodium and chloride net fluxes from I_{sc}:

$$J^{Res}_{net} = I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})$$

Treatments. Lizards were randomly assigned to one of three groups: untreated controls (UC), acutely treated (AT) (which received a single i.p. injection of 100 μg · kg body wt^{-1} D-aldosterone 4 h prior to sacrifice), and chronically treated CT (which received i.p. injections of 100 μg · kg body wt^{-1} D-aldosterone at 52, 42, 28, 18 and 4 h before decapitation). All experiments were performed at the same time of day (16:00 hours) in order to avoid circadian variations in Na transport or plasma aldosterone levels (Clauss et al. 1988). Aldosterone was dissolved in a 50% solution of dimethylsulphoxide (DMSO) in water and immediately administered i.p. as 0.2 ml · kg body wt^{-1}.

Materials. Amiloride, DIDS, ouabain, DMSO and D-aldosterone were purchased from the Sigma Chemical Company (St. Louis Mo., USA).

Statistical procedures. The significance of differences between means was assessed by Student's *t*-test. Treatment means were compared using the unpaired *t*-test or analyses of variance coupled to the Student-Newman-Keuls test. The relationship between I_{sc} and J^{Na}_{m-s} was assessed by means of regression analysis to a linear model followed by a variance analysis. Least-squares linear regression equations are quoted in the figures. A probability value below 0.05 was considered to be significant. Statistical analyses were performed by running the BMDP computer programs (Dixon 1985). Both mathematical and statistical calculations were carried out on an IBM–AT compatible computer. Results are expressed as mean ± SEM and the significance level is indicated in the results. Both I_{sc} and ionic fluxes are expressed in μEq · cm^{-2} · h^{-1}. PD in mV and G_t in mS · cm^{-2}.

Results

Effect of aldosterone treatment on the lizard colon

Electrical parameters in control and aldosterone-treated colons are shown in Fig. 1. Values for PD, I_{sc} or G_t from period II (80–140 min) were not significantly different from values obtained during period I (0–60 min) in UC. AT and CT tissues. Control colons exhibited a low PD and I_{sc} (1.7 ± 0.30 mV and 0.52 ± 0.10 μEq · cm^{-2} · h^{-1}, respectively) being serosal side positive in respect to the mucosal side. Tissue conductance was 11.5 ± 1.6 mS · cm^{-2} in this group of tissues. Acute or chronic aldosterone treatments markedly increased PD and I_{sc} with regard to untreated colons (AT tissues: PD = +204% and I_{sc} = +197%; CT tissues: PD = +172% and I_{sc} = +180%, $P < 0.005$ for all parameters). Increases in PD and I_{sc} were equivalent for both AT and CT tissues and no statistical differences were obtained between them ($P > 0.05$). Tissue conductance was not altered by the steroid in any treatment ($P > 0.05$).

Figure 2 illustrates the results of measurements of unidirectional and net sodium and chloride fluxes in control and aldosterone-treated colons. In UC colons, J^{Na}_{net} = 0.79 ± 0.33 μEq · cm^{-2} · h^{-1}, similar to J^{Cl}_{net}(0.91 ± 0.42 μEq · cm^{-2} · h^{-1}). The net transport of these two ions does not account for I_{sc} and suggests the additional net transport of an undetermined ion. The transport of this ion is given conventionally as J^{Res}_{net}, and was in this case equal to 0.56 ± 0.09 μEq · cm^{-2} · h^{-1}.

Administration of exogenous aldosterone brought about substantial changes in colonic ion transport (Fig. 2). Thus, acute or chronic aldosterone injections resulted in a significant increase in J^{Na}_{net}, which resulted from a rise in the mucosa-to-serosa flux. In spite of different doses of aldosterone administered between AT and CT, no statistical differences between treatments were observed ($P > 0.05$). However, both treatments reduced both J^{Cl}_{m-s} and J^{Cl}_{s-m} with regard to UC colons, but in this case the magnitude of the response between AT and CT was statistically different. In AT tissues J^{Cl}_{net} remained un-

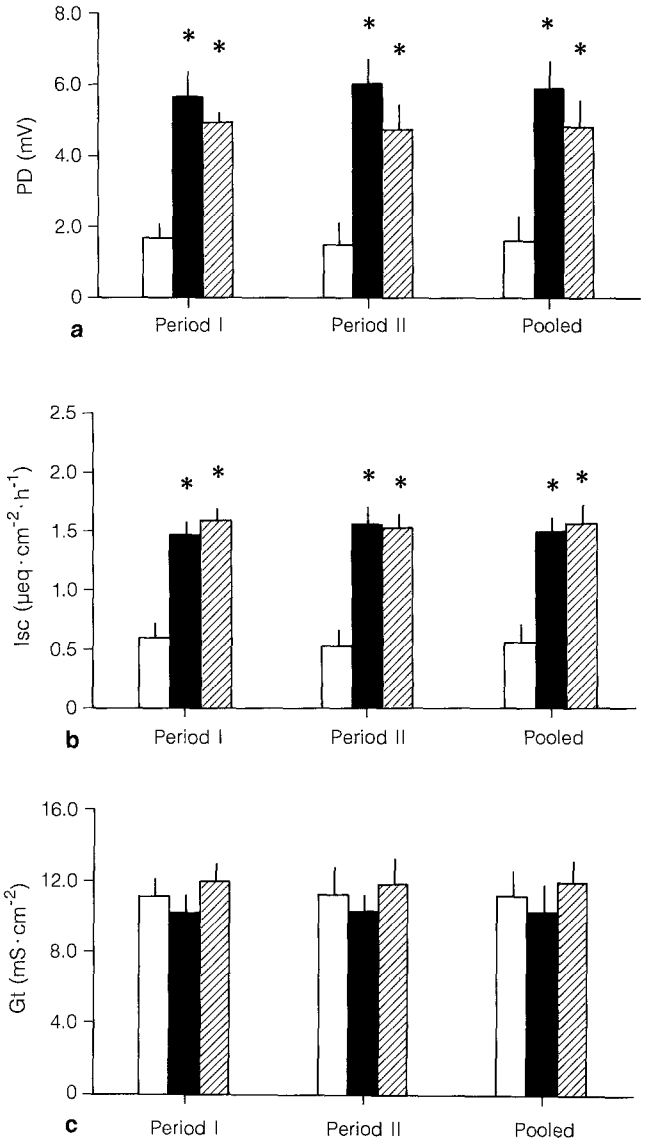

Fig. 1. Bioelectrical parameters in control (UC) and aldosterone-treated (AT and CT) colons: (a) potential difference, (b) short circuit current, (c) tissue conductance. Three 20-min readings were made for each animal/period and averaged to yield a single value for that period. Period I corresponds to the interval 0–60 min and period II to the next 80–140 min (where inhibitors would be added). The number of preparations were 31, 21 and 23 for □ UC, ■ AT and ▨ CT tissues, respectively. $*P<0.005$ between indicated value and corresponding UC-tissues period. No further statistical differences were found between AT and CT tissues

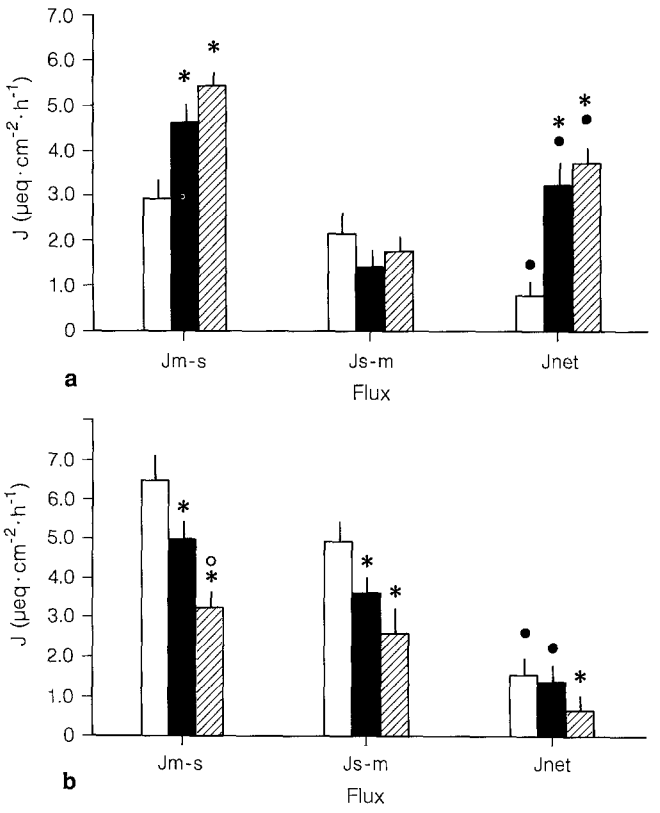

Fig. 2. Unidirectional and net Na^+ and Cl^- fluxes in UC, AT and CT colons. Values are means ± SEM. Three 20-min fluxes were obtained for each tissue and pooled to give a single value. Number of tissues were 11, 12 and 10 for UC □, AT ■ and CT ▨ groups of colons, respectively. $*P<0.05$ with regard to UC tissues; ○ $P<0.05$ with regard to AT-tissues; ● indicates a significant difference from zero at $P<0.05$

changed with regard to UC colons, while J^{Cl}_{net} was completely abolished in CT tissues. This different response to aldosterone was due to the greater reduction of J^{Cl}_{m-s} in CT than in AT tissues ($P<0.05$).

These results strongly suggest that aldosterone, besides increasing Na^+ absorption, also alters colonic Cl^- movements in a dose-dependent fashion. To further explore the changes induced by aldosterone in Na^+ and Cl^- transport, experiments in the presence of pharmacological doses of inhibitors were performed in control and aldosterone-treated lizards.

Effects of amiloride on bioelectrical parameters and NaCl transport across lizard colon

Addition of 10^{-4} *M* mucosal amiloride did not change electrical parameters in UC colons (Table 1) but completely inhibited J^{Na}_{net} ($P<0.05$) and reversed net Cl^- absorption to net secretion ($P<0.05$). This effect was due to decreases in J^{Na}_{m-s} and J^{Cl}_{m-s}. These results are similar to those of previous studies on NaCl absorption in the lizard colon (Badía et al. 1987; Lorenzo et al. 1988; 1990), and have been interpreted as indicating that the mechanism of NaCl absorption in this epithelium is electroneutral and consistent with a double exchange of Na^+ for H^+ and Cl^- for OH^- (HCO_3^-) in the apical membrane of colonic enterocytes.

In contrast to the absence of an effect of amiloride on electrical parameters in control colons, this diuretic significantly decreased aldosterone-induced PD and I_{sc} in AT and CT tissues without altering G_t (Table 1). Likewise, amiloride reduced the aldosterone-increased J^{Na}_{m-s} in AT and CT colons. This reduction of absorptive fluxes caused the inhibition of J^{Na}_{net} (−75.67%) in AT colons but its almost total suppression in CT colons (−93.52%). However, unidirectional and net chloride fluxes were altered by amiloride depending on the treatment (Table 1). Thus, in AT tissues, amiloride inhibited (but did

Table 1. Effects of amiloride (10^{-4} *M*, mucosal) on the electrical properties and NaCl fluxes of control and aldosterone-stimulated colons

	PD	I_{sc}	G_t	J^{Na}_{m-s}	J^{Na}_{s-m}	J^{Na}_{net}	J^{Cl}_{m-s}	J^{Cl}_{s-m}	J^{Cl}_{net}
UC tissues									
Control	1.53±0.32	0.57±0.11	12.73±0.87	2.82±0.17	2.20±0.16	0.62±0.24*	5.36±0.18	4.62±0.28	0.74±0.33*
	12	12	12	8	8	14	6	6	10
+amiloride	1.72±0.21	0.71±0.08	11.88±0.85	2.08±0.19	2.67±0.46	−0.59±0.50	3.40±0.18	4.20±0.23	−0.80±0.29*
	12	12	12	8	8	14	8	8	14
P	NS	NS	NS	<0.05	NS	<0.05	<0.05	NS	<0.01
AT tissues									
Control	4.22±0.44	1.51±0.13	9.78±0.59	4.70±0.23	1.74±0.13	2.96±0.26*	4.71±0.18	3.58±0.18	1.13±0.26*
	11	11	11	7	7	12	7	7	12
+amiloride	1.45±0.18	0.54±0.06	9.68±0.73	2.68±0.23	1.96±0.23	0.72±0.25*	3.73±0.12	3.11±0.28	0.62±0.31*
	11	11	11	9	6	13	7	7	12
P	<0.005	<0.005	NS	<0.01	NS	<0.005	<0.01	NS	<0.05
CT tissues									
Control	4.12±0.31	1.29±0.09	10.34±0.59	5.18±0.31	1.78±0.17	3.40±0.36*	3.17±0.23	2.98±0.26	0.18±0.35
	11	11	11	8	8	14	8	8	14
+amiloride	1.79±0.21	0.67±0.06	10.11±1.29	1.70±0.40	1.48±0.33	0.22±0.52	3.40±0.27	3.34±0.63	0.06±0.69
	8	8	8	6	6	10	5	5	8
P	<0.005	<0.005	NS	<0.005	NS	<0.005	NS	NS	NS

Values are means ± SEM. Numbers below means are the sample size, except for the net fluxes where numbers of degrees of freedom are indicated. *P*, Difference between control and amiloride periods.

* A significant net flux, different from zero, at a probability value <0.05. I_{sc} and ionic fluxes are given in $\mu Eq \cdot cm^{-2} \cdot h^{-1}$: PD in mV, and G_t in $mS \cdot cm^{-2}$. NS, Not significant

not abolish) J^{Cl}_{net} by reducing J^{Cl}_{m-s}, while in CT tissues (where no net Cl^- transport was present) no additional effects were observed. The fact that amiloride reduced I_{sc} and J^{Na}_{net} in parallel in aldosterone-treated tissues strongly suggests that the increased I_{sc} was mainly attributable to an electrogenic Na^+ absorption.

Effects of DIDS

The disulphonic stilbene DIDS, as well as its derivative agent (SITS), are well-known blockers of the anion exchanger in the plasma membrane of red cells (Cabantchik and Rothstein 1972) and of epithelial cells (Emmer and Duffey 1983). To further differentiate the changes induced by aldosterone in the colonic NaCl transport we next examined the effects of DIDS on unidirectional and net Na^+ and Cl^- fluxes in normal and hyperaldosteronized animals. The results of these experiments are shown in Table 2. In UC tissues, mucosal DIDS did not change electrical PD or G_t ($P>0.05$), but completely inhibited J^{Na}_{net} ($P<0.01$) and reversed net Na^+ absorption to net secretion ($P<0.01$), in agreement with the increased I_{sc}.

In AT tissues, this inhibitor of the Cl^-/HCO_3^- exchange reduced J^{Na}_{net} by 71.73% and abolished J^{Cl}_{net}. These responses were clearly due to the significant reductions of the mucosa-to-serosa unidirectional NaCl fluxes without changing opposite fluxes. No changes of PD or I_{sc} could be detected after DIDS in AT tissues. As a net Na^+ absorption significantly different from zero was present after DIDS incubation and no changes in I_{sc} could be observed, we further studied the relationship between Na^+ flux and fractional I_{sc}. It seemed preferable to analyse the parallel evolution of J^{Na}_{m-s} and I_{sc}, since both parameters could be simultaneously obtained in the same tissue, while the net Na^+ flux derives from the calculation of the difference between opposite unidirectional fluxes which necessarily had to be measured in different animals. Naturally, some scatter was expected because of individual variation when assigned to the several experimental conditions. Results from this analysis are illustrated in Fig. 3. A good relationship between I_{sc} and J^{Na}_{m-s} (ISC: $-0.86+0.99\ J^{Na}_{m-s}$, correlation coeficient $=+0.98$) was observed in AT tissues after DIDS, indicating that over a range of J^{Na}_{m-s} values I_{sc} is a function of Na^+ absorption. Thus, in spite of the inhibition of Na^+ transport by DIDS, a fraction of the total Na^+ transport in AT colons is presumably conductive and electrogenic, which could be unmasked by the luminal addition of DIDS. In contrast with the results in AT tissues, incubation with luminal DIDS did not alter unidirectional or net Na^+ or Cl^- fluxes, PD, I_{sc} or G_t in CT tissues (Table 2).

Combined effects of mucosal DIDS and amiloride in AT tissues

Since in the AT group of colons a net Na^+ absorption was present, and no changes in I_{sc} were observed after incubation with mucosal DIDS, suggesting the persistence of the electrogenic Na^+ pathway, experiments were performed to determine the response to amiloride.

In these experiments, the effects of the mucosal addition of both 10^{-3} *M* DIDS and 10^{-4} *M* amiloride (D+A) were analyzed in the group of AT colons. The results of these experiments (Table 3) show that the

Table 2. Effects of DIDS (10^{-3} *M*, mucosal) on the electrical properties and NaCl fluxes of control and aldosterone-stimulated colons

	PD	I_{sc}	G_t	J^{Na}_{m-g}	J^{Na}_{s-m}	J^{Na}_{net}	J^{Cl}_{m-s}	J^{Cl}_{s-m}	J^{Cl}_{net}
UC tissues									
Control	1.45±0.20	0.58±0.14	12.64±1.01	3.01±0.26	1.95±0.26	1.06±0.37*	5.70±0.22	4.22±0.24	1.48±0.32*
	10	10	10	6	6	10	6	6	10
+DIDS	1.83±0.24	0.92±0.09	12.19±1.67	1.38±0.11	1.72±0.11	−0.34±0.16	3.40±0.20	4.82±0.59	−1.41±0.72*
	10	10	10	6	6	10	6	6	10
P	NS	< 0.05	NS	<0.005	NS	< 0.01	<0.01	NS	< 0.01
AT tissues									
Control	5.43±0.55	1.62±0.12	9.43±0.99	5.35±0.46	1.87±0.34	3.48±0.57*	4.71±0.28	3.72±0.33	0.99±0.43*
	11	11	11	6	6	10	6	6	10
+DIDS	6.42±0.24	1.84±0.23	10.56±1.51	2.97±0.28	1.96±0.29	1.01±0.41*	2.85±0.13	3.22±0.12	−0.37±0.32
	11	11	11	6	6	10	6	6	10
P	NS	NS	NS	<0.05	NS	< 0.01	<0.005	NS	< 0.01
CT tissues									
Control	4.81±1.05	1.57±0.25	11.71±2.23	4.93±0.27	2.02±0.31	2.91±0.41*	3.22±0.77	2.76±0.36	0.46±0.65
	10	10	10	6	6	10	6	6	10
+DIDS	5.49±0.31	1.77±0.22	11.20±1.73	4.91±0.37	1.87±0.19	3.04±0.42*	3.62±0.61	3.26±0.25	0.36±0.66
	10	10	10	6	6	10	9	6	12
P	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are means ± SEM. Numbers belows means are the sample size, except for the net fluxes where numbers of degrees of freedom are indicated. *P*, Difference between control and DIDS periods.

* A significant net flux, different from zero, at a probability value <0.05. I_{sc} and ionic fluxes are given in μEq · cm^{-2} · h^{-1}; PD in mV, and G_t in mS · cm^{-2}. NS, not significant

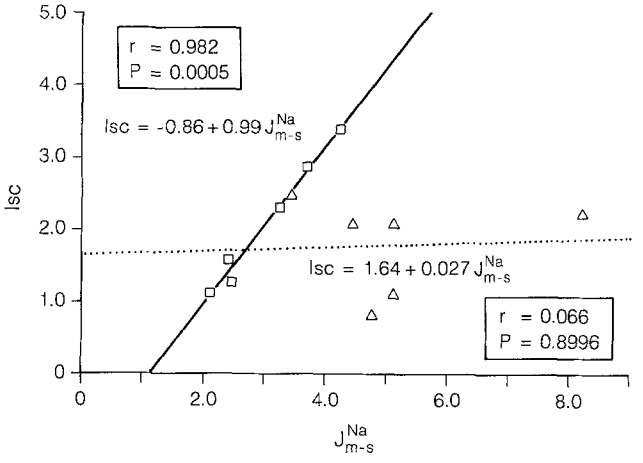

Fig. 3. Relation between J^{Na}_{m-s} and I_{sc} as dependent variable in the group of AT-colons before and after incubation with mucosal 10^{-3} *M* DIDS. In period I (control...△...), a weak slope of 0.027 ± 0.20 (P = 0.89) and an intercept of 1.64 ± 1.11 μEq · cm^{-2} · h^{-1} was present, indicating an apparent independence of I_{sc} and J^{Na}_{m-s}. After luminal addition of DIDS (period II, □), I_{sc} increased as J^{Na}_{m-s} with a significant slope of 0.99 ± 0.094 (P < 0.005), the intercept being −0.86 ± 0.29 μEq · cm^{-2} · h^{-1}, demonstrating that a greater portion of increased I_{sc} in AT tissues occurred by an electrogenic Na^+ absorption

combined effect of these two drugs was to abolish J^{Na}_{net} as the result of a significant reduction of J^{Na}_{m-s} (73.26%) since J^{Na}_{s-m} remained unaltered. In addition, the reduction of net Na^+ absorption was accompanied by a decrease of I_{sc} to near zero (P < 0.05). Thus, no linear relationship between I_{sc} and J^{Na}_{m-s} was present after D+A (data not shown).

Table 3. Effects of simultaneous mucosal addition of DIDS (10^{-3}*M*) and amiloride (10^{-4}*M*, serosal) on Na fluxes and I_{sc} of acutely aldosterone-stimulated colons

	J^{Na}_{m-s}	J^{Na}_{s-m}	J^{Na}_{net}	I_{sc}
Control	5.80±0.77	1.33±0.12	4.46±0.31*	1.41±0.11
	6	6	10	12
DIDS+ amiloride	1.53±0.69	1.63±0.57	−0.10±0.39	0.36±0.15
	6	6	10	12
P	<0.005	NS	< 0.005	< 0.01

Values are means ± SEM. Numbers below means are the sample size except for the net fluxes, where numbers of degrees of freedom are indicated. *P*, Difference between control and inhibitors periods. * A significant net flux, different from zero, at a probability value below 0.05 I_{sc} and ionic fluxes are expressed as μEq · cm^{-2} · h^{-1}. NS, Not significant

Effects of ouabain

The results of incubating normal or aldosterone-treated lizard colons in the presence of serosal 10^{-4} *M* ouabain is shown in Table 3. In untreated lizards, this glycoside inhibited both Na^+ and Cl^- absorption, indicating that both processes depend on the activity of the basolateral Na-K-ATPase. Serosal ouabain significantly reduced I_{sc} and PD, and increased G_t.

In aldosterone-treated tissues, ouabain reduced PD and I_{sc}, increasing G_t. In AT colons, addition of ouabain to the incubating media brought about the total suppression of both Na^+ and Cl^- net fluxes (Table 4). This effect was due solely to the significant reduction of mucosa-to-serosa Na^+ and Cl^- fluxes. In CT tissues, serosal ouabain totally abolished J^{Na}_{net} by reducing J^{Na}_{m-s} without

Table 4. Effects of ouabain ($10^{-4}M$, serosal) on the electrical properties and NaCl fluxes of control and aldosterone-stimulated colons

	PD	I_{sc}	G_t	J^{Na}_{m-s}	J^{Na}_{s-m}	J^{Na}_{net}	J^{Cl}_{m-s}	J^{Cl}_{s-m}	J^{Cl}_{net}
UC tissues									
Control	1.35±0.58	0.51±0.25	12.40±1.34	3.22±0.19	2.14±0.20	1.08±0.28*	5.63±0.21	4.38±0.18	1.45±0.28*
	10	10	10	6	6	10	6	6	10
+ouabain	−0.14±0.24	−0.32±0.13	19.23±1.44	1.66±0.11	1.24±0.16	0.42±0.20	2.24±0.31	1.91±0.18	0.33±0.36
	10	10	10	6	6	10	6	6	10
P	< 0.005	< 0.01	<0.01	<0.01	<0.05	< 0.05	<0.005	<0.005	< 0.05
AT tissues									
Control	4.12±0.32	1.64±0.12	10.94±0.49	5.21±0.36	1.85±0.23	3.36±0.43*	4.46±0.28	3.53±0.20	0.93±0.34*
	10	10	10	6	6	10	6	6	10
+ouabain	1.22±0.32	0.57±0.15	15.29±0.95	1.41±0.32	1.23±0.25	0.18±0.41	3.20±0.51	3.60±0.56	−0.40±0.76
	10	10	10	6	6	10	6	6	10
P	< 0.005	< 0.005	<0.005	<0.005	<NS	< 0.005	<0.05	<NS	< 0.01
CT tissues									
Control	3.12±0.32	0.93±0.07	11.34±0.44	5.19±0.14	1.74±0.11	3.45±0.19*	3.25±0.29	3.26±0.38	−0.01±0.48
	10	10	10	6	6	10	6	6	10
+ouabain	0.88±0.32	0.37±0.09	15.29±1.06	1.89±0.18	2.22±0.21	−0.33±0.28	3.59±0.53	4.19±0.42	−0.60±0.68
	10	10	10	6	6	10	6	6	10
P	< 0.005	< 0.005	<0.005	<0.005	NS	< 0.005	NS	NS	NS

Values are means±SEM. Numbers below means are the sample size, except for the net fluxes where numbers of degrees of freedom are indicated. *P*, Difference between control and ouabain periods.

* A significant net flux, different from zero, at a probability value <0.05 I_{sc} and ionic fluxes are given in $\mu Eq \cdot cm^{-2} \cdot h^{-1}$: PD in mV, and G_t: $mS \cdot cm^{-2}$. NS=not significant

altering J^{Na}_{s-m}, but in contrast to AT or UC colons, neither J^{Cl}_{m-s} nor J^{Cl}_{s-m} were altered by the glycoside.

Discussion

As reported many times in the literature, aldosterone alters electrolyte transport across colonic epithelia (Frizzell and Schultz 1978; Thomas and Skadhauge 1979; Will et al. 1981; Foster et al. 1983). Recent studies have demonstrated that hyperaldosteronism not only stimulates amiloride-sensitive electrogenic Na^+ absorption but also supresses the electroneutral NaCl transport present in the distal colon of untreated rats (Foster et al. 1983; Perrone and Jenks 1984; Halevy et al. 1986). Until recently, it was thought that although aldosterone acted on "tight" epithelia to enhance Na^+ transport, this hormone had no effect on Na^+ transport in more "leaky" epithelia, such as gallbladder or small intestine. Furthermore, it had been thought that only "tight" epithelia had an electrogenic amiloride-sensitive Na^+ transport system, which is on the apical membrane of intestinal cells. However, there is some indirect evidence to suggest that the small intestine may be responsive to aldosterone (Will et al. 1985; Grubb and Bentley 1987). Previous studies performed in our laboratory have shown that the lizard colon exhibits the classical properties of "leaky" epithelia, i.e. low PD and I_{sc}, high G_t and relatively high unidirectional Na^+ and Cl^- fluxes compared to net movements. In a recent paper, we have pointed out that an electroneutral NaCl absorption mediated by parallel Na^+/H^+ and Cl^-/HCO_3^- exchanges is present in control lizard colon (Badía et al. 1987).

The results of the present experiments demonstrate that aldosterone stimulates J^{Na}_{net} and I_{sc}, inducing or activating an electrogenic Na^+ transport. These experiments also indicate that aldosterone-induced Na^+ transport does not depend on the type of treatment administered. Both AT and CT stimulated J^{Na}_{m-s}, J^{Na}_{net} and I_{sc} by a similar amount (Fig. 1). Equivalent natriferic responses at markedly different plasma concentrations of this steroid have been previously observed in the lizard (Díaz and Lorenzo 1992). However, the effect on Cl^- transport was totally distinctive between treatments. Under acute treatment, both J^{Cl}_{m-s} and J^{Cl}_{s-m} were reduced but net Cl^- transport was not altered with regard to UC colons. These results suggest that in these animals the mechanism of double antiports ($Na^+/H^+ - Cl^-/HCO_3^-$) placed in the apical membrane of colonocytes is inhibited but not abolished after acute aldosterone therapy; hence, the mechanism may coexist simultaneously with the electrogenic Na^+ absorption. However, under chronic treatment, tissues exhibited no net Cl^- absorption, indicating that the electroneutral NaCl transport was totally suppressed by the chronic therapy. Evidence for this hypothesis arises from the subsequent experiments performed with amiloride, DIDS and ouabain.

Amiloride has been described as an inhibitor of the Na^+/H^+ antiport for a wide variety of epithelia (Frelin et al. 1988) and as an inhibitor of Na channels in the apical membrane of epithelial cells (Bentley 1968; Cuthbert and Shum 1974; Frelin et al. 1988). In many species, such amiloride-sensitive Na^+ transport can be induced by the adrenocorticosteroid hormone aldosterone (Cuthbert et al. 1979). The data presented in this paper indicate that amiloride decreased aldosterone-induced I_{sc} and J^{Na}_{net} in the colon of AT and CT lizards to control values, showing that the increased Na^+ absorption is responsible

for I_{sc}. Amiloride completely abolished J^{Na}_{net} in CT tissues, whereas in AT colons a significant Na^+ absorption was still present, suggesting that a fraction of Na^+ transport in acutely stimulated colons is not responsible for I_{sc} and is therefore electroneutral. As no net Na^+ absorption persists in the chronic group, such a transport must be absent in CT colons; therefore, the amiloride-sensitive system transporting Na^+ in untreated colons must be completely suppressed in CT colons but only partially inhibited in AT tissues.

This hypothesis is also supported by the results of measurement of Cl^- fluxes. Indeed, amiloride inhibited, but did not abolish, net Cl^- absorption in AT tissues (as a consequence of the significant reduction of J^{Cl}_{m-s}), but was without effect in CT tissues. This is in agreement with the suppression of electroneutral NaCl absorption after chronic therapy.

In addition, the present results suggest that the Na^+/H^+ exchange is sensitive to this low dose of amiloride in control conditions but not in acutely stimulated colons. It seems possible that amiloride exhibits a higher affinity by the conductive Na process than by the electroneutral Na^+/H^+ antiport. This hypothesis is supported by reported results demonstrating that the concentration of amiloride (10^{-4} *M*) used is more effective in inhibiting the electrogenic Na^+ absorption by blocking apical Na^+ channels (Zeiske et al. 1982) rather than the electroneutral Na^+/H^+ antiport (Frelin et al. 1988). Thus, assuming the coexistence of both absorptive mechanisms in AT tissues, the response to 10^{-4} *M* amiloride is mainly due to the inhibition of the electrogenic process.

The results obtained when DIDS was added to the mucosal reservoir suggest that this stilbene blocks the activity of Cl^-/HCO_3^- and, indirectly, the coupled antiporter Na^+/H^+ on colonic enterocytes of UC and AT tissues. This drug inhibited mucosa-to-serosa Na^+ and Cl^- fluxes in both tissues and, as a consequence of this effect, DIDS abolished J^{Cl}_{net} in AT colons; however, J^{Na}_{net} was only reduced (not abolished) to a similar extent as J^{Cl}_{net}. Moreover, following DIDS addition to AT tissues, I_{sc} remained unchanged despite the reduced net Na^+ fluxes. A good relationship between I_{sc} and J^{Na}_{m-s} could then be established, probably because of the eliminated electroneutral Na^+ absorption, suggesting that after suppression of coupled NaCl absorption, the remaining Na^+ absorption is conductive and electrogenic. These results are in agreement with the existence of a positive relationship between I_{sc} and J^{Na}_{m-s} in control CT tissues which could be suppressed by luminal amiloride (Díaz and Lorenzo 1991), and with the fact that DIDS exerted no effect either on I_{sc} or on Na^+ and Cl^- fluxes in CT colons; this is to be expected since chronic therapy suppresses electroneutral NaCl absorption.

The addition of both DIDS and amiloride to the mucosal reservoir clearly demonstrates that both absorptive processes are present at the mucosal surface of AT colon; net Na^+ flux was completely abolished and I_{sc} was reduced to near zero, which would be expected considering additive effects of both DIDS and amiloride on the electroneutral and electrogenic Na^+ absorption, respectively.

The abolishing of net Na^+ and Cl^- fluxes by ouabain (Table 4) clearly indicates that NaCl transport in UC tissues depends on the activity of basolateral ATPase activity. The analysis of net electrolyte transport before and after the addition of the glycoside in the group of AT colons revealed that both electroneutral and electrogenic Na^+ transport systems exist after AT, since ouabain not only reduced Na^+ transport but also Cl^- transport (Table 4). However, in CT tissues ouabain abolished Na^+ transport and I_{sc} but did not alter Cl^- fluxes, which is consistent with the suppression of coupled NaCl transport by chronic aldosterone treatment. These results are in good agreement with those published by Perrone and Jenks (1984) and Halevy et al. (1986) demonstrating that aldosterone suppresses coupled NaCl absorption in the distal rat colon.

The reasons for the differences between AT and CT are not yet understood. However, a possible explanation could be related to the differences in plasma levels of aldosterone that have been previously observed (Díaz and Lorenzo 1992). Indeed, plasma concentration of aldosterone in CT was about six-fold that in UC, and two-fold that in AT. Recent studies indicate that the inhibition of electroneutral NaCl absorption may be a function of the magnitude of the increase in plasma aldosterone levels (Halevy et al. 1986). In rats with an excess of dietary potassium, which is associated with a ten-fold increase in plasma aldosterone levels, both amiloride-sensitive electrogenic Na^+ transport and active K^+ secretion were induced, but electroneutral NaCl absorption was not altered (Budinger et al. 1986). However, Halevy et al. (1986) demonstrated that progressive aldosterone infusion for 24, 48 and 72 h to rats produced varying changes in distal colon ion transport; electrogenic Na^+ absorption progressively increased, whereas electroneutral NaCl was initially augmented but then inhibited. Our observations indicate that the inhibition of electroneutral NaCl absorption requires higher levels of plasma aldosterone than does the stimulation of electrogenic Na^+ channels. Thus, it is clear that the electroneutral process might coexist at least temporarily with the conductive Na^+ absorption in the colonic mucosa.

In summary, our experiments demonstrate that aldosterone alters NaCl transport in "leaky" epithelia. There is also evidence that the effect of this steroid is influenced by the magnitude of the elevation of plasma levels. Thus, lower doses are sufficient to stimulate electrogenic Na^+ absorption (which may coexist with the electroneutral process), while higher levels are required to suppress electroneutral NaCl absorption.

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