Comp. Biochem. Physiol. Vol. 100A, No. 1, pp. 63–68, 1991
Printed in Great Britain

0300-9629/91 $3.00 + 0.00

REGULATION OF AMILORIDE-SENSITIVE Na^+ ABSORPTION IN THE LIZARD (*GALLOTIA GALLOTI*) COLON BY ALDOSTERONE*

M. Díaz† and A. Lorenzo

Laboratorio de Fisiología Animal, Departamento de Biología Animal. Universidad de La Laguna, 38206 Tenerife, Canary Islands, Spain

(*Received* 2 *November* 1990)

Abstract—1. Acute (AT) or chronic (CT) administration of exogenous aldosterone brought about a considerable increase in transmural potential difference (PD), short-circuit current (Isc) and net sodium flux (J^{Na}_{net}).

2. A good relationship between Isc and J^{Na}_{m-s} was observed in CT but not in AT or UC (untreated controls).

3. Addition of mucosal amiloride reduced Isc in AT and CT colons, but did not cause any change in UC colons. The relationship between Isc and J^{Na}_{m-s} observed in CT was totally suppressed by the diuretic.

4. J^{Na}_{net} was reduced in AT tissues and abolished in CT colons by amiloride.

5. The present results strongly suggest that aldosterone levels quantitatively and qualitatively modify sodium absorption across colonic mucosa in a dose-dependent fashion and that lower levels of the hormone are required to induce the electrogenic Na absorption than to suppress the electroneutral transport.

INTRODUCTION

Previous studies performed in our laboratory have shown that the lizard colon exhibits the classical properties of "leaky" epithelia (i.e. low potential difference and short-circuit current, high tissue conductance and relatively high unidirectional Na and Cl fluxes compared to net movements). In this epithelium, the predominant sodium absorptive transport appears to be an electroneutral mechanism mediated by the presence of an amiloride-sensitive, Na^+–H^+ exchange process coupled to a Cl^-/HCO_3^- antiport, in the apical membrane of colonocytes (Badía *et al.*, 1987).

Aldosterone stimulates Na absorption, K secretion and fluid absorption in a wide variety of tissues (see Sandle and Binder, 1987, for review). Until quite 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 was thought that only "tight" epithelia had an electrogenic amiloride-sensitive Na transport system, located in the apical membrane of intestinal cells. In many species such amiloride-sensitive Na channels can be induced by the adrenocorticoid hormone aldosterone (Cuthbert *et al.*, 1979; Will *et al.*, 1985b). Nevertheless, it has been demonstrated that Na depletion in rats resulted in the appearance of amiloride-sensitive Isc, presumed to reflect Na transport in the ileum, an effect that was apparently mediated by aldosterone (Will *et al.*, 1985a). The aim of the present experiments was to examine the response of the lizard colon to acute or chronic i.p. aldosterone injections using a completely *in vitro* preparation under voltage-clamp conditions. In addition, we focused our experiments on the relation of aldosterone treatment to natriferic response and the sensitivity of basal and mineralocorticoid-induced Isc and J^{Na} to inhibition by amiloride.

MATERIALS AND METHODS

Collection of animals and tissue preparation

Adult male and female *Gallotia galloti* lizards, trapped in Tenerife (Canary Islands, Spain), were transported to the laboratory and acclimated in a large indoor terrarium for 2–4 days before being used for experimental purposes. Mean body weight of experimental animals was 39.34 ± 1.23 g. Water was provided *ad libitum*. 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 solution, which was used as the bathing medium, contained (in mM), 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 mM. Additionally, solutions were gassed with a mixture of 95% CO_2–5% O_2, resulting in a pH of 7.4 at 30°C, the temperature at which experiments were performed.

Tissue incubation, electrical measurements and flux determination

All studies were performed using standard Ussing-type chambers (0.21 cm^2 exposed surface area). After mounting the tissue, 4 ml of Ringer solution were added to both mucosal and serosal sides. Solutions were circulated by gas lift and thermostatically kept at 30°C in water-jacketed half-chambers. Mucosal amiloride was added to the respective reservoir in small volumes from concentrated stock solutions to obtain a final bath concentration of 10^{-4} M.

*A preliminary report of this work was presented at the 24th meeting of the Sociedad Española de Ciencias Fisiológicas, Madrid, Spain. September 1990.

†Requests for reprints should be addressed to: Dr Mario Díaz at the above address.

This dose was chosen because it has been widely shown to be effective in inhibiting the electrogenic Na transport and because we previously observed that it could abolish the Na^+/H^+ antiport in the lizard colon (Badía *et al.*, 1987).

Each tissue chamber was connected to an automatic computer-controlled voltage-clamp device (AC-microclamp. Aachen, F.R.G.) that allowed continuous measurement of the short circuit current (Isc) electrical potential difference (PD) and tissue conductance (Gt) as reported previously (Díaz and Lorenzo, 1989).

Approximately 5 μCi of ^{22}Na were 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_{m-s}) and serosa-to-mucosa (J_{s-m}) fluxes were calculated from two 200 μl aliquots taken every 20 min during an initial 60 min flux period (pre-amiloride) and a second 60 min flux period after the addition of the loop diuretic (post-amiloride) from the unlabelled side. Comparisons of drug effects and their corresponding controls were carried out as pre-amiloride versus post-amiloride periods. The activity of ^{22}Na in the flux samples was determined by using a β-counter. 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, 1989). The net flux was calculated as the difference between oppositely directed unidirectional fluxes ($J_{net} = J_{m-s} - J_{s-m}$).

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 weight D-aldosterone 4 hr prior to blood sampling, and chronically treated (CT), which received i.p. injections 100 μg/kg body weight at 52, 42, 28, 18 and 4 hr prior to the experiment. Aldosterone (D-aldosterone. Sigma) was dissolved in a 50% solution of dimethylsulfoxide (DMSO) in water and immediately administered. The administered volume was adjusted to be 0.2 ml solution/kg body weight. Since it has been demonstrated that circadian oscillations of endogenous aldosterone plasmatic levels clearly reflect variations of colonic transport characteristics (Clauss *et al.*, 1988), all experiments were performed at the same time (4 pm).

Mathematical and statistical procedures

Sensitivity to mucosal amiloride in the various treatments was evaluated with two parameters: IS (relative amiloride-sensitive current) and JS (relative amiloride-sensitive J^{Na}_{m-s}):

$$JS = 1 - \frac{J^{Na}(\text{post-amiloride})}{J^{Na}(\text{pre-amiloride})}$$

and

$$IS = 1 - \frac{\text{Isc (post-amiloride)}}{\text{Isc (pre-amiloride)}}$$

Both variables range between 1 and $-\infty$. IS and JS tend to 1 when maximal inhibition is produced (i.e. total suppression of Isc or J^{Na}_{m-s}), while a 0 value for these parameters indicates no effect, and values below 0 indicate stimulation.

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 short-circuit current and J^{Na}_{m-s} was assessed by means of regression analysis to a linear model followed by an analysis of variance. Least-squares linear regression equations are quoted in Table 1 with intercepts (i), slopes (s), standard error of estimates (SE), the correlation coefficient (r) and the significance levels of r (P). Slopes of regression lines were compared by covariance analysis. A probability value (P) 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 a Digital Vax/Vms computer. Results are expressed as mean ±SEM and the significance level is indicated in the results.

Drugs

Amiloride, DMSO and D-aldosterone were purchased from the Sigma Chemical Co. (St Louis, MO).

RESULTS

Effects of exogenous aldosterone on the electrical activity and Na *transport in the lizard colon*

The effects of acute or chronic aldosterone therapies on *in vitro* electrical parameters and sodium fluxes across colonic preparations are summarized in Table 2. The values of Isc, PD and Gt for normal colons were comparable to those previously reported (Badía *et al.*, 1987; Lorenzo *et al.*, 1987, 1990). Aldosterone augmented PD and Isc with regards to UC ($P < 0.005$, for both AT and CT procedures), but no differences in PD, Isc or Gt were observed between AT and CT epithelia ($P > 0.05$ in all cases). No statistical differences could be observed in Gt means between normal and aldosterone-treated tissues ($P > 0.05$).

The rise in colonic PD and Isc in aldosterone-treated colons suggest the induction of electrogenic sodium absorption. In order to test this hypothesis, unidirectional and net Na^+ fluxes were measured in normal and aldosterone-stimulated colons (Table 2). The data revealed that in standard KRB colonic preparations of normal lizards actively absorbed sodium at a rate of 0.79 μeq/cm^2 hr. Aldosterone treatment significantly increased J^{Na}_{net}, this increase being due to a rise in J^{Na}_{m-s} (Table 2). To study more precisely the relationship between Isc and sodium fluxes, the parallel behaviour of J^{Na}_{m-s} and Isc was analysed, since both parameters could be simul-

Table 1. Linear regression equations between Isc and J^{Na}_{m-s} as independent variable, in the UC (a), At (b) and CT (c) experimental groups before and after incubation with luminal amiloride

Group	Period	y-Intercept	Slope	r	SE	P
(a) UC	pre-amiloride	0.028 ± 0.77	−0.182 ± 0.22	−0.31	0.486	0.440
	post-amiloride	0.523 ± 0.47	−0.050 ± 0.18	−0.11	0.286	0.794
(b) AT	pre-amiloride	0.960 ± 0.17	+0.025 ± 0.03	+0.38	0.135	0.455
	post-amiloride	1.217 ± 0.29	−0.109 ± 0.08	−0.55	0.086	0.255
(c) CT	pre-amiloride	0.033 ± 0.39	+0.271 ± 0.08	+0.85	0.153	0.029
	post-amiloride	0.769 ± 0.04	−0.087 ± 0.02	−0.88	0.051	0.019

r = correlation coefficient; SE = standard error of estimation; P = probability level from the analysis of variance.

Table 2. Electrical properties and unidirectional and net sodium fluxes across isolated lizard colon in control (UC), acutely-stimulated (AT) and chronically-treated (CT) groups of colons incubated in standard KRB and with 10^{-4} M mucosal amiloride

	PD (mV)	Isc (μeq/cm^2 hr)	Gt (mS/cm^2)	J^{Na}_{ms} (μeq/cm^2 hr)	J^{Na}_{s-m} (μeq/cm^2 hr)	J^{Na}_{net} (μeq/cm^2 hr)
UC						
Standard	1.58 ± 0.37	0.56 ± 0.14	11.27 ± 0.63	2.93 ± 0.22	2.14 ± 0.25	0.79 ± 0.33‡
	31	31	31	18	15	31
†Amiloride	1.40 ± 0.32	0.52 ± 0.08	11.88 ± 0.85	2.08 ± 0.19†	2.67 ± 0.46	−0.50 ± 0.50†
	12	12	12	8	8	14
AT						
Standard	4.75 ± 0.63***	1.50 ± 0.12***	10.35 ± 0.58	4.63 ± 0.54***	1.80 ± 0.25	3.22 ± 0.21***‡‡‡
	23	23	23	12	13	23
†Amiloride	1.45 ± 0.18†††	0.54 ± 0.06†††	9.99 ± 0.73	2.68 ± 0.23††	1.96 ± 0.23	0.72 ± 0.25†††‡
	11	11	11	9	6	15
CT						
Standard	4.81 ± 1.05***	1.60 ± 0.25***	10.34 ± 1.04	5.45 ± 0.25***	1.73 ± 0.25	3.71 ± 0.61***‡‡‡
	23	23	23	18	18	34
†Amiloride	1.79 ± 0.21†††	0.58 ± 0.06†††	10.11 ± 1.29	1.70 ± 0.40†††	1.48 ± 0.33	0.22 ± 0.52†††
	11	11	11	8	8	14

Values are expressed as mean ± SEM. The numbers below each mean ± SEM correspond to the number of determinations. *Significant differences between indicated values and corresponding UC value. †Differences between amiloride and standard KRB period. ‡Differs significantly from zero. *, † or ‡ $P < 0.05$, **, †† or ‡‡ $P < 0.01$; ***, ††† or ‡‡‡ $P < 0.005$.

taneously obtained in the same tissue while the net sodium flux derives from the calculation of the difference between opposite unidirectional fluxes, which necessarily had to be measured in different animals. As can be seen in Table 1, no relationship between Isc and J^{Na}_{m-s} was present in UC ($P > 0.05$). In spite of the equivalent response of the lizard colon to acute or chronic aldosterone, regression analyses of J^{Na}_{m-s} on Isc revealed different patterns for AT and CT animals (Table 1). Indeed, CT induces a positive correlation between Isc and J^{Na}_{m-s}, indicating that over a range of values, Isc is a function of J^{Na}_{m-s}. However, such a relationship was indistinguishable in AT animals. At this stage, it appears that unlike the findings in CT, increased Isc in AT colons seems to be unrelated to J^{Na}_{m-s}.

Effects of amiloride

The ability of amiloride to inhibit both Isc and sodium absorption was examined in the three groups of tissues (Table 2). Amiloride (10^{-4} M) was added to the mucosal bathing solution at the end of period I (pre-amiloride) and its effects were observed at the end of the second period (post-amiloride). In control tissues, amiloride abolished J^{Na}_{net} ($P < 0.05$), but did not change Isc. These results are in good agreement with our previous findings (Badía *et al.*, 1987; Lorenzo *et al.*, 1990) on this same tissue, indicating the electrically silent nature of sodium absorption under basal conditions. In steroid-treated tissues, amiloride reduced Isc and J^{Na}_{m-s} but did not change J^{Na}_{s-m}. The magnitude of the reduction was dependent on the type of aldosterone treatment. Thus, in AT tissues amiloride reduced J^{Na}_{net} by 77.64% and Isc by 64%, but a significantly different from zero net Na absorption was present despite the reduction of Isc to control values, suggesting that some electroneutral Na absorption is yet present after amiloride. On the other hand, in CT colons J^{Na}_{net} and Isc were much more altered by the diuretic than in AT tissues, since J^{Na}_{net} was totally abolished (−94.07%) and Isc was reduced, as in AT tissues, to control values (−63.75%), indicating that aldosterone-induced Isc was totally abolished by the diuretic, and that no electrogenic sodium absorption persists following amiloride.

To further explore the effects of amiloride, the parallel evolution of relative amiloride-sensitive Isc and amiloride-sensitive J^{Na}_{m-s} in the three groups of tissues was analysed. The results of double scatterplot in Fig. 1 show that as long as the exogenous administration is augmented, the points are closer to one another and nearer to the point 1,1 (maximal inhibition), indicating that longer exposures to aldosterone increase amiloride-sensitive Isc and J^{Na}_{m-s}.

Regression analysis of Isc on J^{Na}_{m-s} before and after amiloride addition is shown in Table 1. Significant changes were only observed in CT tissues. Amiloride significantly altered the relationship between J^{Na}_{m-s} and Isc in CT tissues, reversing the positive correlation ($r = 0.85$, $P < 0.05$) during the pre-amiloride period to a negative value ($r = -0.88$, $P < 0.05$). The y-intercept of regression lines in CT tissues (Table 1), before and after amiloride were significantly different, i.e. in the pre-amiloride period the intercept was zero (0.03 ± 0.39), while in the post-amiloride period it was 0.77 ± 0.04. As unidirectional serosa-to-mucosa fluxes were not statistically changed by amiloride, the difference between the y-intercept and J^{Na}_{s-m} should be zero if the only ionic species involved in the generation of Isc was sodium ion. As already explained in the Materials and Methods, simultaneous measurements of J^{Na}_{ms} and J^{Na}_{s-m} were not possible by the employed methods, but an alternative approach to this calculation (intercept minus J^{Na}_{s-m}) could be made by using mean J^{Na}_{s-m}. The computed results of these differences were −1.7 and −0.7 for the pre- and post-amiloride periods, respectively, which indicated the presence of an active ionic transport other than sodium. Several reasons suggest that the best candidate to explain these observations is the ion K^+ (see Discussion).

The fact that aldosterone in AT colons increases both Isc and J^{Na}_{m-s} without any apparent relationship, and that the reduction of Isc after amiloride is due to the reduction of the electrogenic Na absorption, points out that a non-conductive Na absorption is also present in the AT, but absent in the CT tissues. To further investigate this point, the relationship between amiloride-inhibitable Isc and total Isc in AT and CT groups of colons was analysed. The results from this analysis (Fig. 2) indicate that both in AT

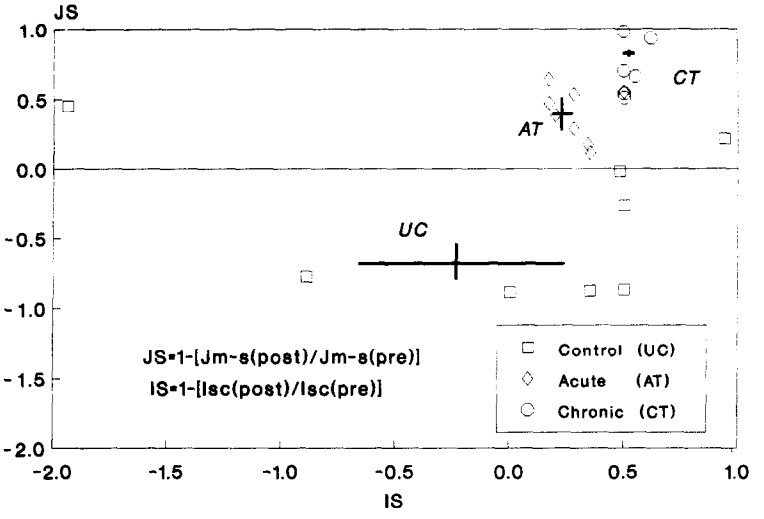

Fig. 1. Double scatterplot of IS (amiloride-sensitive Isc) versus JS (amiloride-sensitive J^{Na}_{m-s}) for the three experimental conditions. Lengths of lines are proportional to SEM.

and CT colons, amiloride-inhibitable Isc was also correlated with total Isc. The slope of relationship in CT colons was greater and better correlated than that induced by AT ($P < 0.05$), demonstrating that a larger portion of the aldosterone-induced Isc occurred by an amiloride-sensitive pathway in the CT treatment than in the acute treatment.

DISCUSSION

A variety of studies have shown that hyperaldosteronism (produced by the administration of exogenous aldosterone or secondary to dietary sodium depletion) increases colonic sodium and water absorption, as well as potassium secretion (see Sandle and Binder, 1987). In the rat distal colon, hyperaldosteronism not only stimulates an amiloride-sensitive electrogenic Na transport but also inhibits the electroneutral sodium chloride transport present in untreated rats (Foster *et al.*, 1983; Perrone and Jenks, 1984).

Until recently, such aldosterone-induced amiloride-sensitive sodium transport has only been observed in "tight" epithelia (with relatively high electrical resistance). However, an amiloride-sensitive Isc could be observed in the *in vitro* small intestine from animals with high mineralocorticoid levels (Grubb and Bentley, 1987; Will *et al.*, 1985a). This is interesting since the small intestine is recognized as a "leaky" epithelium and small intestine NaCl absorption has been characterized as an electrically silent process (Will *et al.*, 1985a).

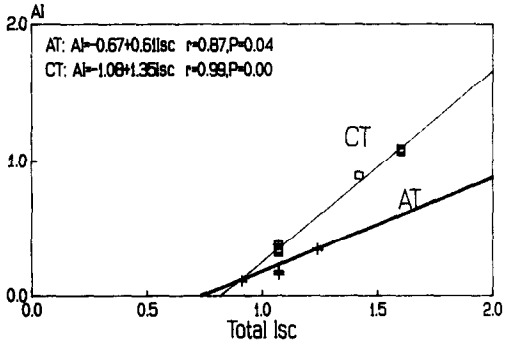

Fig. 2. Relationship between amiloride-inhibitable Isc (AI) and total Isc in AT (crosses) and CT (squares) tissues. AI values were obtained as the difference between basal value and response value obtained after adding the drug.

The purpose of the present study was to examine the effects of aldosterone on sodium transport across lizard colon, a "leaky" epithelium in which NaCl absorption is mediated by a system of double exchange Na^+/H^+–Cl^-/HCO_3^-. We have previously observed that acute or chronic exogenous aldosterone administration is associated with increased circulating hormone levels in the lizard, being the maximal levels reached with the chronic treatment (Díaz and Lorenzo, in press).

The results presented in this paper indicate that both acute and chronic aldosterone therapies increase Isc and net sodium flux, an effect which was due to a rise in J^{Na}_{m-s}, suggesting that the hormone had induced or activated an electrogenic Na absorption. The results also show that the response to acute aldosterone differ from that of chronic treatment, since a significant relationship between Isc and J^{Na}_{m-s} was only present in CT but not in AT tissues (Table 1), indicating that only after chronic treatment, Isc is a function of J^{Na}_{m-s}.

Amiloride decreased aldosterone-induced Isc in the colon of AT and CT lizards to control values, showing that the increased absorptive Na fluxes are responsible for Isc. The appearance of maximal amiloride-sensitive Isc requires a much longer period of exposure to aldosterone (i.e. chronic treatment) since the evolution of J^{Na}_{m-s} along the treatments shown in Fig. 1 indicates that Na^+ is more inhibited by amiloride the longer the exposures to aldosterone. However, 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 Isc and hence is electroneutral. As no net Na

absorption persists in the chronic group, such a transport is absent in CT colons, and therefore, the amiloride-sensitive system transporting sodium of untreated colons must have been completely suppressed in CT colons but partially inhibited in AT tissues.

The reasons for which the Na^+/H^+ exchange is sensible to this low dose of amiloride in control conditions but not in acutely-stimulated colons are not yet clear. It seems probable that amiloride exhibit a higher affinity by the conductive Na process than by the electroneutral Na^+/H^+ antiport. This conclusion is supported by reported results demonstrating that the concentration of amiloride assayed by us better inhibit the electrogenic Na absorption by the blocking of apical Na^+ channels (Zeiske *et al.*, 1982) 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 may be due to the inhibition of the electrogenic process. The positive correlation between amiloride-inhibitable Isc and total Isc observed in AT and CT tissues (Fig. 2) support the notion that in all treatments aldosterone-induced Isc is due to the induction (or activation) of electrogenic sodium channels.

The results presented in this paper also indicate that although the aldosterone-stimulated preparations exhibited increases in Isc and PD, no changes could be detected in the tissue conductance, which usually increases in high-resistance membranes exposed to the hormone. In addition, aldosterone-treated tissues responded to amiloride with declines in Isc and PD, but no changes were observed in conductance, which usually decreases in "tight" epithelia exposed to the drug. This is not perhaps surprising considering the high basal conductance of the control tissues and the variability observed by us. Similar results have been found in the avian (Grubb and Bentley, 1987) and mammalian small intestine (Will *et al.*, 1985a).

Nevertheless, the analyses also shown that aldosterone-induced Isc does not totally account for the increase in J^{Na}_{net} since the rise of Isc was lower than J^{Na}_{net} in aldosterone-treated animals, and one must propose the additional net transport of an undetermined ion. Many studies have revealed that aldosterone also induces the appearance of an active potassium secretion (Foster *et al.*, 1983, 1984; Kliger *et al.*, 1981) and, in a previous report, we were able to demonstrate that aldosterone induced a barium-sensitive amiloride-insensitive K^+ secretion by stimulating J^{K}_{s-m} in the lizard colon (Díaz and Lorenzo, 1990). The observed potassium secretion of -0.99 ± 0.11 μeq/cm^2 hr is in agreement with the intercepts (-0.67 ± 0.061 μeq/cm^2 hr) of the regression lines for amiloride-sensitive Isc and total Isc in AT tissues. A similar situation was present in CT tissues, where the rate of transcellular potassium secretion was -1.23 ± 0.19 μeq/cm^2 hr, very close to the intercept -1.089 ± 0.28 μeq/cm^2 hr of amiloride-sensitive Isc versus total Isc. These findings might explain the fact that J^{Na}_{net} was higher than Isc in AT and CT tissues.

It has been shown that in epithelia such as amphibian skin, mammalian colon and urinary bladder, which have been studied widely *in vitro*, aldosterone stimulates Isc and Na transport in 1–2 hr and that these responses involve a process of genetic transcription and translation and the appearance of *de novo* proteins, generically named AIP (aldosterone-induced proteins) (Fanestil and Kipnowski, 1982). The latter are thought to either promote the formation of new Na channels or activate channels that are already present in the plasma membrane. The effects on the lizard colon, require chronic exposure to aldosterone to achieve the maximum response. As pointed out by Grubb and Bentley (1987), aldosterone possibly promotes a morphological transformation or differentiation of new cells containing amiloride-sensitive Na channels. Thus, it is clear that, at least for a period of time, the electroneutral process might coexist with the conductive absorption in the colonic mucosa.

In conclusion, our experiments strongly suggest that aldosterone induces an amiloride-sensitive electrogenic sodium absorption in the lizard colon, an effect that requires lower doses of aldosterone than those needed to suppress electroneutral Na^+/H^+ absorption. However, additional experiments using pharmacological tools, including specific analogues of amiloride, should be performed to characterize the model of NaCl transport in aldosterone-stimulated lizard colon.

REFERENCES

Badía P., Lorenzo A., Díaz M. and Gómez T. (1987) Mechanism of transport of Na and Cl in the lizard colon. *Comp. Biochem. Physiol.* **87A**, 883–887.

Clauss W., Dürr J. E., Krattenmacher R., Hörnicke H. and Van Driessche, W. (1988) Circadian rhythm of apical Na-channels and Na-transport in rabbit distal colon. *Experientia* **44**, 608–610.

Cuthbert A. W., Edwardson J. M., Aceves J. and Wilson S. A. (1979) Amiloride as a membrane probe. In *Amiloride and Epithelial Sodium Transport* (Edited by Cuthbert A. W., Ganelli G. M. and Scriabine A.), pp. 113–123. MD: Urban and Schwarzengerg. Baltimore.

Díaz M. (1989) Cálculo computarizado de flujos iónicos transmembranarios. *Tecn. Lab.* **146**, 445–458.

Díaz M. and Lorenzo A. (1989) Modulation of active potassium transport by aldosterone. *Comp. Biochem. Physiol.* **95A**, 79–85.

Díaz M. and Lorenzo A. Plasma electrolyte and aldosterone concentrations in the lizard *Gallotia galloti* (Sauria. Lacertidae). *Herpetol. J.* (in press).

Dixon W. J. (1985) BMDP Biomedical computer programs. 734 pp. Univ. California Press. Berkeley.

Fanestil D. D. and Kipnowski J. K. (1982) Molecular action of aldosterone. *Klin. Wochenschr.* **60**, 1180–1185.

Frelin Ch., Barbry P., Chassande O. and Vigne P. (1988) Amiloride-sensitive Na^+ transport systems. In *Molecular Basis of Biomembrane Transport* (Edited by Palmieri F. and Quagliariello E.), pp. 81–90. Elsevier, Amsterdam.

Foster E. S., Zimmerman T. W., Hayslett J. P. and Binder H. J. (1983) Corticosteroid alteration of active electrolyte transport in rat distal colon. *Am. J. Physiol.* **245**, G668–G675.

Foster E. S., Hayslett J. P. and Binder H. J. (1984) Mechanism of active potassium absorption and secretion in the rat colon. *Am. J. Physiol.* **246**, G611–G617.

Grubb B. and Bentley P. J. (1987) Aldosterone-induced, amiloride-inhibitable short-circuit current in the avian ileum. *Am. J. Physiol.* **253**, G211–G216.

Kliger A. S., Binder H. J., Bastl C. and Hayslett J. P. (1981) Demonstration of active potassium transport in the mammalian colon. *J. Clin. Invest.* **67**, 1189–1196.

Lorenzo A., García T., Rodríguez A., Gómez T. and Badía P. (1987) Effect of theophyline on ion transport in the lizard colon. *Comp. Biochem. Physiol.* **88A,** 405–410.

Lorenzo A., Medina V., Badía P. and Gómez T. (1990) Effects of vasopressin on electrolyte transport in the lizard intestine. *J. Comp. Physiol. B* **159,** 745–751.

Perrone R. D. and Jenks S. L. (1984) Suppression of coupled Na–Cl absorption by aldosterone and dexamethasone in rat distal colon. *Am. J. Physiol.* **246,** F785–F793.

Sandle G. I. and Binder H. J. (1987) Corticosteroid and intestinal ion transport. *Gastroenterology* **93,** 18–196.

Schultz S. G. and Zaluski P. (1964) Ion transport in isolated rabbit ileum—II. The interaction between active sodium transport and active sugar transport. *J. Gen. Physiol.* **48,** 1043.

Will P. C., Cortright R. N., Groseclose R. G. and Hopfer U. (1985a) Amiloride-sensitive salt and fluid absorption in small intestine of sodium-depleted rats. *Am. J. Physiol.* **248,** G124–G132.

Will P. C., Cortright R. N., Delisle R. C., Douglas J. G. and Hopfer U. (1985b) Regulation of amiloride-sensitive electrogenic sodium transport in the rat colon by steroid hormones. *Am. J. Physiol.* **248,** G133–G141.

Zeiske W., Wills N. K. and Van Driessche W. (1982) Na^+ channels and amiloride-induced noise in the mammalian colon epithelium. *Biochim. Biophys. Acta.* **688,** 201–210.