J. exp. Biol. ***186****, 9–22 (1994)*
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EFFECTS OF BARIUM CHLORIDE ON ELECTROLYTE TRANSPORT ACROSS ISOLATED COLONS FROM NORMAL AND ALDOSTERONE-TREATED LIZARDS (*GALLOTIA GALLOTI*)

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Accepted 31 August 1993

Summary

Addition of $BaCl_2$ to the solution bathing colons of normal lizards did not alter the absorptive Na^+ flux, but did reverse the net absorption of Cl^- to become net secretion. Cl^- secretion resulted from an increase in its serosal-to-mucosal movement and was positively correlated to an increase in short-circuit current. Ba^{2+}-induced short-circuit current was dependent on the presence of both Na^+ and Cl^- in the serosal medium. Ba^{2+}-induced Cl^- secretion could be reversed by serosal amiloride (10^{-4} mmol l^{-1}). Colons from acutely or chronically aldosterone-treated lizards exhibited a considerably higher short-circuit current, potential difference and net Na^+ absorption than did untreated colons. Net Cl^- transport was unaltered by acute treatment, but was totally abolished after chronic treatment. $BaCl_2$ rapidly decreased the potential difference, short-circuit current and tissue conductance across colons from aldosterone-treated lizards. Net Na^+ transport was markedly inhibited by Ba^{2+} in both acutely and chronically treated tissues, but barium did not change unidirectional or net Cl^- fluxes in these. The present results support the following hypotheses: (1) that $BaCl_2$ inhibits electrogenic Na^+ absorption induced by acute or chronic aldosterone treatment and (2) that Ba^{2+} induces an electrogenic Cl^- secretion by stimulating a basolateral Na^+-dependent Cl^- intake in normal but not in aldosterone-treated colons. This also suggests that aldosterone could exert an antisecretory influence in colonic epithelia.

Introduction

The lizard colon has been shown to absorb Na^+ and Cl^- actively by means of an

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Key words: Ba^{2+}, aldosterone, NaCl transport, electrogenic Na^+ absorption, Cl^- secretion, *Gallotia galloti*.

electroneutral mechanism involving a double exchange of Na^+/H^+ and Cl^-/HCO_3^- (Badía *et al.* 1987). Moreover, the existence of active Cl^- secretion across normal lizard colons has been recently observed (Díaz and Lorenzo, 1991*a*). This process is electrogenic, depends on the uptake of Cl^- into the cell across the basolateral membrane and is thought to occur by an electrically neutral NaCl co-transport process. The energy for this transport is derived from the Na^+ gradient, which is maintained by the Na^+ pump. This secretory process can be unmasked in normal lizards by blocking the absorptive fluxes in the presence of the disulphonic stilbene DIDS or the pirazine diuretic amiloride in the mucosal bath, and it is activated by cyclic AMP (Díaz and Lorenzo, 1991*a*) or agents that increase cyclic AMP levels, such as theophylline (Lorenzo *et al.* 1987). The mechanisms responsible for NaCl transport in the lizard colon are affected in a dose-dependent fashion by the mineralocorticoid hormone aldosterone. Cl^- absorption is completely abolished while Na^+ absorption is increased by an electrogenic and non-coupled process if the lizards are chronically treated with aldosterone, whereas acute steroid administration increases electrogenic Na^+ absorption and inhibits, but does not abolish, electroneutral NaCl absorption (Díaz and Lorenzo, 1992). In addition, we have recently observed that a single aldosterone injection induced an electrogenic K^+ secretion that could be blocked by mucosal barium (Díaz and Lorenzo, 1990), but Ba^{2+} blockade was not followed by an increase in the short-circuit current as would be expected for unmasked electrogenic Na^+ absorption. Therefore, alterations of ionic movements other than a simple blockade of K^+ transport should take place after Ba^{2+} exposure. Recently, a chloride secretory process stimulated by $BaCl_2$, and consistent with an increase in intracellular Ca^{2+} concentration, has been demonstrated in the rat small intestine and colon (Hardcastle *et al*. 1983, 1985). Our present study was designed to investigate the effects of $BaCl_2$ on NaCl transport across the lizard colon before and after acute or chronic aldosterone infusions. Our aims were first to determine whether electrogenic Cl^- secretion can be elicited by barium in the lizard colon and then to investigate how aldosterone regulates this transport.

Materials and methods

Animals, solutions and tissue preparation

Adult male and female lizards *Gallotia galloti* (Sauria: Lacertidae) weighing 36.43±2.13 g were captured in Punta del Hidalgo (Tenerife) and acclimatized in a large indoor terrarium for 2–4 days before being used for experimental purposes. Lizards were randomly assigned to one of three groups: untreated controls (UC); acutely treated (AT) animals, which received a single intraperitoneal injection of 100 μg kg^{-1} body mass D-aldosterone 4 h prior to being killed; and chronically treated (CT) animals, which received intraperitoneal injections of 100 μg kg^{-1} body mass D-aldosterone 52, 42, 28, 18 and 4 h before decapitation. All experiments were performed at the same time (16:00 h) to avoid circadian variations in Na^+ transport or plasma aldosterone levels (Clauss *et al.* 1988*b*). Aldosterone was dissolved in a solution containing 50 % dimethylsulphoxide (DMSO) in normal Ringer (vehicle solution, see below) and immediately administered as

0.2 ml kg^{-1} body mass per injection. UC animals received a single injection of vehicle solution.

The standard Ringer's 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; D-(+)glucose, 12. Additionally, solutions were gassed with a mixture of 5 % CO_2 and 95 % O_2, resulting in a pH of 7.4.

Colons were removed from decapitated lizards, opened along their mesenteric border, rinsed free of luminal contents and immersed in ice-cold Ringer's solution. Tissues were mounted in standard Ussing-type chambers (0.21 cm^2 exposed surface area). 4 ml of Ringer's solution was added to both mucosal and serosal sides. Solutions were regulated at 25 °C. Serosal solution was isosmotically adjusted with D-(+)mannitol when $BaCl_2$ was added to the mucosal reservoir. When Na^+-free or Cl^--free solutions were used, the corresponding ion was replaced with choline or isethionate, respectively. Progressive addition of Na^+ or Cl^- in ion-substitution experiments was performed by replacing 0.2 ml of both mucosal and serosal bathing solutions by an equal volume of a normal Ringer's solution.

Electrical measurements

Agar bridges (4 % w/v in 3 mol l^{-1} KCl) were positioned near each of the tissues and at opposite ends of the chamber. Calomel electrodes and Ag/AgCl electrodes in saturated KCl were connected by the agar bridges to measure the transepithelial potential difference and to pass direct current, respectively. 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 (Lorenzo *et al*. 1990).

Flux measurements

Flux determinations were initiated after a minimum period of 30 min because preliminary observations indicated that stable flux rates were achieved within 30 min of isotope addition (^{22}Na or ^{36}Cl) to either the mucosal or serosal bath. Unidirectional mucosal-to-serosal (J_{ms}) and serosal-to-mucosal (J_{sm}) fluxes were calculated from two 200 ml samples taken every 20 min from the unlabelled side during an initial 60 min flux period (control) and a second 60 min flux period after the addition of Ba^{2+}. The activity of radioisotopes was determined in a β-counter (LKB-1209, Rackbeta). Unidirectional Na^+ and Cl^- fluxes were calculated according to standard equations from Schultz and Zalusky (1964) with a computer program developed in our laboratory (Díaz and Cozzi, 1991).

The net flux was calculated as:

$$J_{net} = J_{sm} - J_{sm}.$$

Net residual flux was calculated by subtracting the difference between Na^+ and Cl^- net fluxes:

$$J_{net}^{Res} = I_{sc} - (J_{net}^{Na} - J_{net}^{Cl}).$$

Statistical procedures

Results are expressed as means ± S.E.M. Differences between treatments were assessed using analysis of variance. The significance of differences between means was tested using a two-tailed Student's *t*-test. The relationship between short-circuit current and J_{sm}^{Cl} was assessed by means of linear regression analysis followed by a variance analysis. A probability value below 0.05 was considered to be significant. Statistical analyses were performed using BMDP computer programs (Dixon, 1985).

Materials

DMSO (dimethylsulphoxide), DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid), amiloride and D-aldosterone were purchased from Sigma. Radioisotopes (^{22}Na and ^{36}Cl) were obtained from New England Nuclear.

Results

Effects of $BaCl_2$ on normal lizard colons

Untreated colons exhibited a low PD and I_{sc} (1.62±0.38 mV and 13.93±2.68 μA cm^{-2}, respectively), the serosal side being positive with respect to the mucosal side. Tissue conductance was 11.25±1.46 mS cm^{-2}.

Addition of $BaCl_2$ (5 mmol l^{-1}) to the bathing solution caused a sustained increase in I_{sc} from 16.08±4.29 to 31.36±5.03 μA cm^{-2} (P<0.01; Fig. 1A). This increase in I_{sc} was accompained by a rise in G_t from 9.10±0.97 to 12.11±0.36 mS cm^{-2} (P<0.01). There was only a small insignificant increase in PD (1.81±0.34 *versus* 2.06±0.44 mV).

Unidirectional flux measurements revealed that net Na^+ absorption was similar to net Cl^- absorption and that the residual flux (J_{net}^{Res}) was almost zero. Addition of mucosal $BaCl_2$ altered neither net Na^+ transport nor residual flux (Table 1). However, Ba^{2+} caused a significant change in Cl^- transport, i.e. Cl^- absorption was reversed to net secretion, this effect being due to the increase in J_{sm}^{Cl} (+40.13 %, P<0.01) because J_{ms}^{Cl} was not altered.

The finding that the changes in net Cl^- transport were associated with those of I_{sc} strongly suggests that net Cl^- secretion is electrogenic. Fig. 1B illustrates the least-squares regression lines for normal colons before and after Ba^{2+} addition. The slope of the regression for the control period ($I_{sc}=-0.64+0.24J_{sm}^{Cl}$) was not significantly different from zero (P=0.35) but, after luminal addition of Ba^{2+}, I_{sc} increased as J_{sm}^{Cl} with a significant slope of 0.57±0.12 (P=0.011): $I_{sc}=-2.39+0.5J_{sm}^{Cl}$. As can be seen, under control conditions I_{sc} bore no relationship to the serosal-to-mucosal Cl^- flux. In contrast, following Ba^{2+} treatment, I_{sc} increased as a function of J_{sm}^{Cl}, indicating not only that Ba^{2+} stimulated transcellular J_{sm}^{Cl} but also that such Cl^- movement was electrogenic, i.e. I_{sc} increased as Cl^- secretion was augmented.

In a previous paper (Lorenzo *et al.* 1987) we reported that theophylline-induced Cl^- secretion was electrogenic, dependent on serosal Na^+ and probably mediated by a basolateral Na^+/H^+, Cl^-/HCO_3^- double-antiport mechanism. To explore the influence of barium on the colonic Cl^- transport described here, two different sets of experiments

Table 1. *Effects of $BaCl_2$ (5 mmol l^{-1} mucosal) on the electrical properties and Na^+ and Cl^- fluxes of lizard colons*

	I_{sc}	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	J^{Res}_{net}
Untreated (UC)								
Control	0.59±0.16	2.93±0.22	2.14±0.25	0.79±0.33*	5.47±0.14	4.56±0.17	0.91±0.42*	0.48±0.23
	12	6	6	10	6	6	10	10
+Ba^{2+}	1.16±0.18	3.04±0.29	2.36±0.13	0.68±0.13*	5.29±0.30	6.39±0.21	−1.10±0.37*	0.36±0.26
	12	6	6	10	6	6	10	10
P	<0.005	NS	NS	NS	NS	<0.005	<0.005	NS
Acutely treated (AT)								
Control	1.49±0.13†	4.63±0.54†	1.40±0.25	3.23±0.21*†	4.60±0.26†	3.63±0.23†	0.97±0.40*	−0.77±0.19†
	12	6	6	10	6	6	10	10
+Ba^{2+}	0.50±0.13	2.24±0.26	1.53±0.22	0.71±0.35*	4.71±0.35	3.97±0.22	0.74±0.41*	0.53±0.21
	12	6	6	10	6	6	10	10
P	<0.005	<0.05	NS	<0.01	NS	NS	NS	<0.05
Chronically treated (CT)								
Control	1.69±0.11†	5.45±0.25†	1.73±0.25	3.72±0.61*†	3.24±0.25†	2.77±0.39†	0.47±0.45	−1.55±0.26†
	10	6	6	10	6	6	10	10
+Ba^{2+}	0.80±0.09	2.65±0.68	2.16±0.49	0.49±0.42	3.25±0.38	3.13±0.38	0.12±0.54	0.43±0.23
	10	6	6	10	6	6	10	10
P	<0.005	<0.05	NS	<0.005	NS	NS	NS	<0.01

Values are means ± S.E.M.
Numbers below means are the sample size.
P, difference between control and Ba^{2+} periods.
*A significant net flux different from zero at $P<0.05$.
†Significantly different from untreated colons ($P<0.05$).
Results are expressed as μequiv cm^{-2} h^{-1}.
NS, not significant.

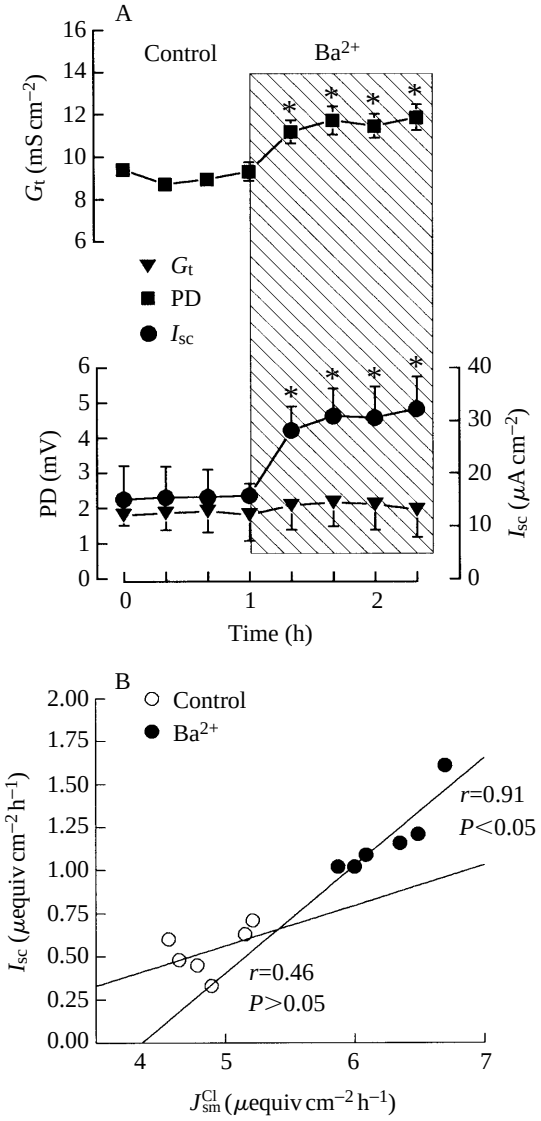

Fig. 1. (A) Time course of the changes in potential difference (PD), short-circuit current (I_{sc}) and tissue conductance (G_t) across normal (UC) colons before (control) and after (Ba^{2+}) addition of mucosal $BaCl_2$ (5 mmol l^{-1}). Each point represents the mean ± S.E.M. for 11 experiments. (Value significantly different ($P<0.05$) from the control value at 1 h. (B) Relationship between J^{Cl}_{sm} and I_{sc} in the group of untreated lizard colons before and after addition of 5 mmol l^{-1} mucosal Ba^{2+}.

were performed. First, the ionic dependence of barium-induced Cl^- secretion was assessed in ion-replacement experiments and, second, serosal amiloride (10^{-4} mmol l^{-1}) was used to test whether Na^+/H^+ antiport might be directing Cl^- uptake through the basolateral membrane. When ion-substitution experiments were performed, the tissues

were first incubated in Na^+-free or Cl^--free bathing solution to obtain the baseline values of I_{sc}. Once a steady state had been reached, 5 mmol l^{-1} $BaCl_2$ was added to both mucosal and serosal solutions and, after a new steady state had been reached, ion replacements were started. As shown in Fig. 2A, progressive addition of Cl^- to the bathing medium brought about a continued increase in I_{sc} in Ba^{2+}-stimulated, but not in unstimulated, tissues, indicating that increased I_{sc} was dependent on Cl^- in the incubation medium. A similar effect on I_{sc} was observed when Na^+ was substituted by choline. As can be seen in Fig. 2B, a concentration-dependent, saturable increase in I_{sc} was present in Ba^{2+}-stimulated, but not in control, tissues. A half-maximal effect on I_{sc} was seen at approximately 24 mmol l^{-1} Na^+ and a maximal effect at 96 mmol l^{-1} Na^+. Similarly a half-maximal effect on I_{sc} was seen at 60 mmol l^{-1} Cl^- and a maximal effect at 105 mmol l^{-1} Cl^-, indicating that the maximal Ba^{2+}-induced I_{sc} is reached with a lower Na^+ than Cl^- concentration. In the second approach, the effects of serosal amiloride on Ba^{2+}-induced Cl^- secretion were assayed. Fig. 2C summarizes the results of these experiments. As expected, Ba^{2+} induced a significant Cl^- secretion due to an increase in J^{Cl}_{sm}. Subsequent addition of 10^{-4} mmol l^{-1} serosal amiloride reversed the net secretion to net absorption mainly by reducing J^{Cl}_{sm}.

Effects of $BaCl_2$ on the colon of aldosterone-treated lizards

Acute or chronic aldosterone treatments markedly increased PD and I_{sc} compared with those of untreated colons, but did not change G_t (compare Figs 1A and 3). No statistically significant differences between AT and CT tissues were observed for any of the three electrical variables. Acute or chronic exogenous aldosterone injections elicited a significant increase in J^{Na}_{ms} and J^{Na}_{net}, but no statistically significant differences could be detected in Na^+ transport between AT and CT tissues (Table 1). Both treatments reduced J^{Cl}_{ms} and J^{Cl}_{sm} compared with UC colons, but in this case the magnitude of the response between AT and CT colons was statistically different. In AT tissues, net Cl^- transport remained unchanged compared with UC colons, whereas J^{Cl}_{net} was completely abolished in CT tissues. This different response to aldosterone was due to the greater reduction of J^{Cl}_{ms} in CT than in AT tissues ($P<0.05$). These results confirm previous findings about the hormonal regulation of NaCl transport in this species (Díaz and Lorenzo, 1991*b*, 1992).

Mucosal Ba^{2+} decreased PD, I_{sc} and G_t across the colon of both acutely and chronically aldosterone-treated lizards (Figs 3A,B, respectively). The magnitudes of these changes were not significantly different between treatments ($P>0.05$). These changes in colonic electrical activity induced by Ba^{2+} were accompanied by changes in Na^+ and Cl^- movements. In AT tissues, Ba^{2+} significantly decreased the mucosal-to-serosal Na^+ flux, and thus the net Na^+ flux (Table 1), but the net flux remained greater than zero. However, the reduction of J^{Na}_{ms} was not paralleled by a decrease in J^{Cl}_{ms}, suggesting that Ba^{2+} was acting on electrogenic Na^+ absorption rather than on the coupled NaCl transport. After chronic aldosterone treatment (Table 1), $BaCl_2$ reduced Na^+ absorption, as it did in AT colons, but neither unidirectional nor net Cl^- fluxes were altered by Ba^{2+}, a result in agreement with the absence of net Cl^- movements in control CT tissues. In addition, the negative net residual flux observed in control AT and CT tissues was consistent with, and of the same magnitude as, the aldosterone-induced secretory K^+ transport that we have

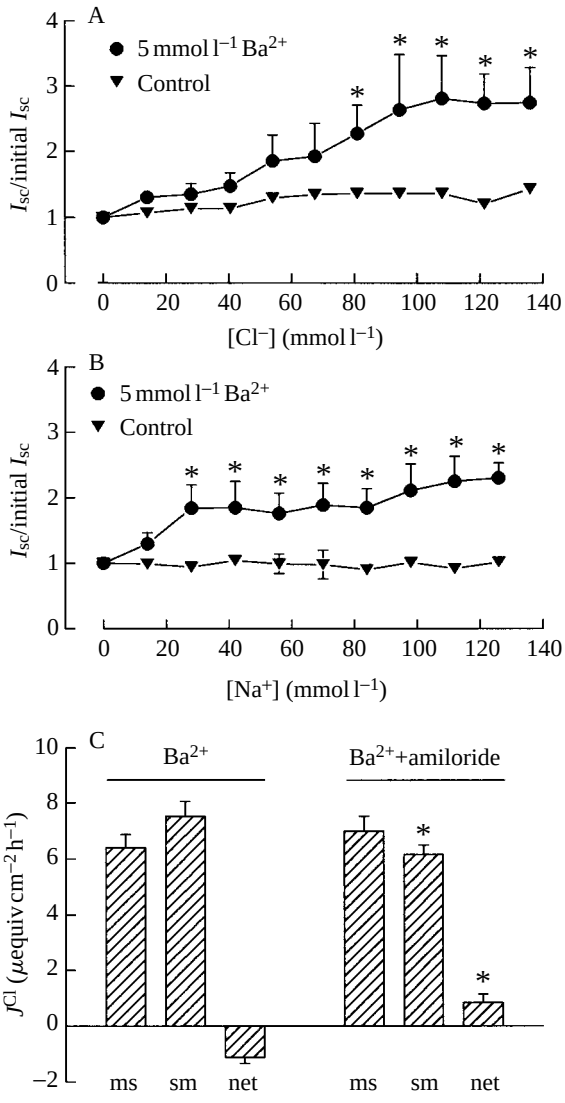

Fig. 2. Dependence of the barium-induced increase in I_{sc} on Cl^- (A) and Na^+ (B) concentration. Tissues were originally bathed on both sides in Cl^--free (A) or Na^+-free (B) solutions. Bathing solutions were then replenished with the appropriate ion simultaneously on both mucosal and serosal sides. A stepwise increase in the ion concentration was obtained by removing an appropriate volume of Na^+-free or Cl^--free solution and then adding the same volume of bathing solution of normal composition. *P<0.05 compared with the corresponding control value. (C) Effects of serosal amiloride (10^{-4} mmol l^{-1}) on Ba^{2+}-induced Cl^- secretion. Tissues were first incubated in the presence of 5 mmol l^{-1} Ba^{2+} and unidirectional and net Cl^- fluxes were measured (Ba^{2+}). Amiloride was then added to the serosal medium and, after 20 min, unidirectional and net Cl^- fluxes were again measured (Ba^{2+}+amiloride). *Value is significantly different (P<0.05) from the corresponding Ba^{2+} value. Net fluxes were significantly different (P<0.05) from zero. ms, mucosal-to-serosal flux; sm, serosal-to-mucosal flux; net, net flux. Values are mean ± S.E.M., N=5.

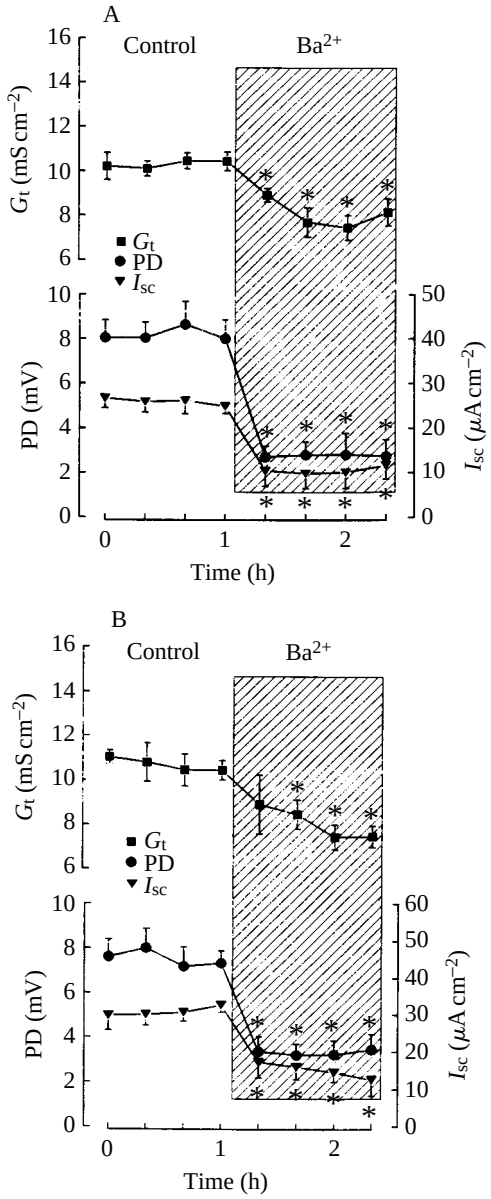

Fig. 3. Changes in potential difference (PD), short-circuit current (I_{sc}) and tissue conductance (G_t) across acutely treated colons (A) and chronically treated colons (B) before (control) and after (Ba^{2+}) mucosal addition of 5 mmol l^{-1} $BaCl_2$. Each point represents the mean ± S.E.M. for 12 and 11 experiments, respectively. *Value is significantly different (P<0.05) from the control value before addition of Ba^{2+}.

previously observed (Díaz and Lorenzo, 1990). Ba^{2+} brought the calculated J_{net}^{Res} towards zero in AT and CT tissues, as would be the case if a net potassium transport were responsible for J_{net}^{Res}.

Discussion

Ba^{2+} has been reported to alter electrolyte and sugar transport activity in intestinal epithelia (Alcalde and Ilundain, 1988; Brown and Sepúlveda, 1985; Harcastle *et al.* 1983, 1985). In stripped sheets of rat mid-intestine and colon, $BaCl_2$ has been shown to increase the serosal-to-mucosal movement of Cl^-, suggesting that it had activated a Cl^- secretory process in response to the Ba^{2+}-induced Ca^{2+} release from intracellular stores (Hardcastle *et al.* 1983, 1985). In addition to its effects on Cl^- transport, in the rat colon but not in the mid-intestine, Ba^{2+} also reduces net Na^+ absorption, mainly by decreasing J^{Na}_{ms}. This effect is not accompanied by a corresponding fall in the mucosal-to-serosal flux, indicating that $BaCl_2$ acts on the electrogenic Na^+ absorption rather than on the coupled NaCl transport that exists in rat colon (Binder and Rawlins, 1973; Charney *et al.* 1981).

The lizard colon can secrete chloride by an electrogenic mechanism that requires Na^+ on the serosal side and that is blocked by serosal ouabain (Díaz and Lorenzo, 1991*a*; Lorenzo *et al.* 1987). There is basal serosal-to-mucosal transport of Cl^-, i.e. a secretory Cl^- flux, across normal lizard colons that is unmasked when the absorptive mucosal-to-serosal flux is selectively inhibited (Díaz and Lorenzo, 1991*a*). $BaCl_2$ added to the mucosal side of the lizard colon increased I_{sc} and G_t and elicited a net Cl^- secretion without altering mucosal-to-serosal Na^+ or Cl^- fluxes or net Na^+ absorption. Cl^- secretion was due solely to an increase in the serosal-to-mucosal flux and was electrogenic, because there was a positive relationship between I_{sc} and J^{Cl}_{sm}. That Cl^- movements were responsible for the increase in I_{sc} was demonstrated in the ion-substitution experiments: in the absence of chloride in the bathing solution, no increase in I_{sc} could be observed and progressive Cl^- addition brought about a saturable increase in I_{sc}. Furthermore, Ba^{2+}-induced Cl^- secretion is dependent on basolateral Na^+ uptake. This conclusion can be drawn because replacement of Na^+ in the bathing solution with choline abolished the rise in I_{sc} induced by Ba^{2+} and because Ba^{2+}-induced Cl^- secretion could be reversed to net absorption by adding amiloride to the serosal bath. Similar reductions in Ba^{2+}-induced I_{sc} by serosal ouabain, a Na^+ pump inhibitor, and furosemide, a loop diuretic, have previously been reported by Hardcastle *et al.* (1985). In addition, because chloride secretion was dependent on the presence of Na^+ in the bathing solution, but barium did not change J^{Na}_{net}, it is inferred that Na^+ is recycled to the serosal medium by the Na^+ pump. This conclusion is supported by the observation that the half-maximal increase in I_{sc} induced by barium can be attained at a lower Na^+ than Cl^- concentration. In summary, these results in the normal lizard colon suggest that the main effect of $BaCl_2$ might be the activation of the secretory process existing at the basolateral membrane in a similar way to the model described by Lorenzo *et al.* (1987) for theophylline. According to this model, a neutral NaCl entry mechanism at the basolateral membrane of colonic enterocytes transports Cl^- into the cell against its electrochemical potential, coupled to the downhill movement of Na^+ into the cell. Na^+ is then recycled out of the cell by the Na^+/K^+-ATPase in the basolateral membranes. Cl^- may then leave the cell at the apical membrane through a conductive pathway, diffusing passively down its electrochemical potential gradient (Fig. 4A).

In the lizard colon, aldosterone induces electrogenic Na^+ absorption and produces

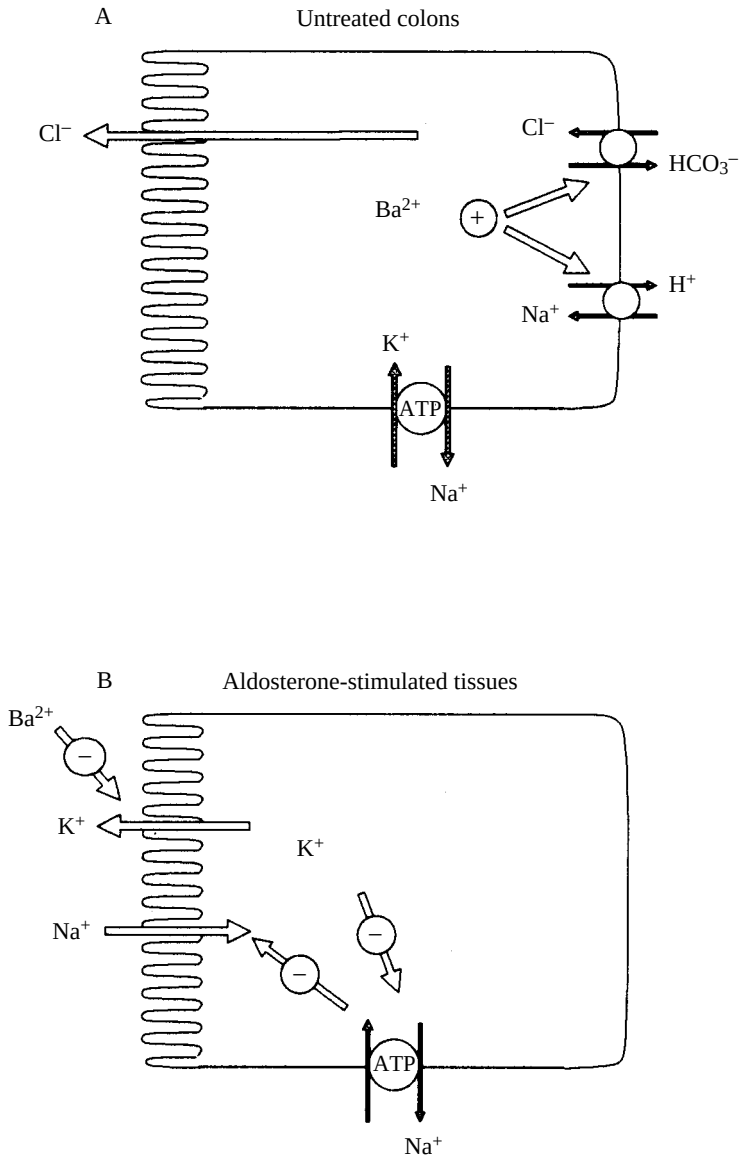

Fig. 4. Hypothetical models for the effects of $BaCl_2$ on colonic NaCl transport across untreated (A) and aldosterone-treated (B) colons. In untreated tissues, Ba^{2+} induces an electrogenic Cl^- secretion by stimulating the activity of basolateral electroneutral NaCl (Na^+/H^+ and Cl^-/HCO_3^-) transport. Na^+ is then recycled through the Na^+ pump, and Cl^- leaves the cell across the apical membrane down its electrochemical gradient. In aldosterone-treated colons, Na^+ is mainly absorbed by an electrogenic Na^+ mechanism. Ba^{2+} inhibits Na^+ absorption indirectly by increasing the intracellular K^+ activity. In aldosterone-stimulated tissues, Ba^{2+} does not induce electrogenic Cl^- secretion. For additional explanation, see Discussion. Circles with + or − symbols indicate positive and negative effects, respectively, on the transport processes indicated by the arrows.

dose-dependent changes in the electroneutral NaCl absorption: a single aldosterone infusion of 100 mg kg^{-1} body mass inhibited electroneutral NaCl absorption, but it co-existed with the electrogenic Na^+ absorption induced by the steroid, whereas pretreatment with several intraperitoneal injections of 100 mg kg^{-1} body mass completely blocked electroneutral absorption while the electrogenic Na^+ transport remained active (Díaz and Lorenzo, 1992). Furthermore, both treatments reduced the serosal-to-mucosal chloride fluxes, indicating a suppression of basolateral Cl^- uptake (Díaz and Lorenzo, 1992). The results presented in Table 1 are consistent with those previously reported by us and support the hypothesis that low doses of aldosterone do not abolish electroneutral NaCl absorption whereas higher doses do, because the effects of $BaCl_2$ on Cl^- and Na^+ transport in aldosterone-treated lizards clearly differ from those in normal lizards. $BaCl_2$ altered neither J^{Cl}_{sm} nor J^{Cl}_{net}. As no net Cl^- secretion appears after Ba^{2+} treatment of either AT or CT tissues, the mechanism of electroneutral NaCl transport in the basolateral membrane must be suppressed by aldosterone, and it appears that, in normal lizards, the stimulation of Cl^- secretion must be mediated by the activation of the double exchange of Na^+/H^+ and Cl^-/HCO_3^- in the basolateral membrane.

Aldosterone has been shown to modulate electrogenic Cl^- secretion induced by theophylline in the colon and coprodeum of *Gallus domesticus* (Clauss *et al.* 1988*a*, 1991; Skadhauge *et al.* 1989). Cl^- secretion induced by theophylline is facilitated by a low-salt diet (which increases plasma aldosterone concentration) and is inhibited by serosal bumetanide, an inhibitor of the $Na^+/2Cl^-/K^+$ co-transporter (Clauss *et al.* 1991). Our results with Ba^{2+} are clearly distinguishable from those in the hen colon, because in the lizard colon Ba^{2+} could not elicit any detectable Cl^- secretion in aldosterone-stimulated tissues. One plausible explanation for this finding is that Ba^{2+} and theophilline stimulate different intracellular pathways that activate Cl^- secretion. Indeed, it is well documented that theophylline inhibits the 3′,5′-phosphodiesterase and therefore increases cyclic AMP levels, whereas barium-dependent secretion does not require any augmentation of this mediator (Hardcastle *et al.* 1983, 1985). In contrast to the lack of effect of Ba^{2+} on Cl^- transport, this cation reduced net Na^+ absorption in aldosterone-treated tissues. This alteration of Na^+ movement in AT and CT colons was due to the reduction in J^{Na}_{ms} and was accompanied by a decrease in I_{sc} and PD, suggesting that $BaCl_2$ was exerting some inhibitory effect on the aldosterone-induced electrogenic Na^+ absorption. Ba^{2+} is known to block K^+ channels in a number of tissues (Hermsmeyer and Sperelakis, 1970; Sheppard *et al*. 1988) and this effect can explain many of its actions. In the normal lizard colon, no net potassium transport exists under control conditions, but primary hyperaldosteronism results in significant potassium secretion, and this active secretory process is selectively abolished by mucosal Ba^{2+} (Díaz and Lorenzo, 1990). The negative J^{Res}_{net} observed in control AT and CT tissues, as well as the observation that this variable is reversed to positive values after barium treatment, is in very good agreement with the induction of an active potassium secretion by aldosterone in the lizard, as reported previously (Díaz and Lorenzo, 1990).

A tentative model accounting for the effects of barium on aldosterone-induced Na^+ transport is shown in Fig. 4B. According to this model, blocking the exit of potassium across the apical membrane may result in an increase in its intracellular activity, which

indirectly reduces the activity of the basolateral Na^+ pump. Thus, the concomitant increase in intracellular Na^+ concentration reduces Na^+ permeability and, consequently, entry of this ion across the apical membrane. This hypothetical model is supported by several published observations in different animals. First, a negative Na^+ feedback between intracellular Na^+ activity and apical permeability was reported by Kirk and Dawson (1985) for the turtle colon. Second, Ba^{2+}-sensitive electrogenic Na^+ transport has been observed in the rat colon (Binder and Rawlins, 1973), rabbit distal colon (Halm and Frizzell, 1986) and turtle colon (Halm and Dawson, 1984*a*,*b*). In conclusion, our experiments indicate, that in the normal lizard colon, Ba^{2+} causes an active Na^+-dependent Cl^- secretion without altering the normal absorptive NaCl process, whereas, in aldosterone-treated colons, Ba^{2+} does not induce Cl^- secretion but reduces aldosterone-induced electrogenic Na^+ absorption.

This study was supported by Research Grant number 24/91 of the Consejería de Educación del Gobierno de Canarias, Spain.

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