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EFFECTS OF CORTICOSTEROID HORMONES ON THE ELECTROPHYSIOLOGY OF RAT DISTAL COLON: IMPLICATIONS FOR Na^+ AND K^+ TRANSPORT

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SUMMARY

1. Conventional microelectrodes, the Na^+ channel blocker amiloride (0·1 mM), and the K^+ channel blocker tetraethylammonium chloride (TEA, 30 mM) were used to examine the effects of corticosteroid hormones administered *in vivo* on the Na^+ and K^+ transport properties of isolated rat distal colon. The cell membrane changes induced by aldosterone (a specific mineralocorticoid), RU 28362 (a synthetic glucocorticoid with negligible affinity for mineralocorticoid receptors), and dexamethasone (an activator of both mineralocorticoid and glucocorticoid receptors) were compared.

2. In control animals, there was no amiloride-sensitive apical Na^+ conductance, and only a relatively small TEA-sensitive apical K^+ conductance.

3. Hyperaldosteronism secondary to dietary Na^+ depletion for 10–14 days, dexamethasone (600 μg 100 g^{-1} day^{-1} for 3 days), and RU 28362 (600 μg 100 g^{-1} day^{-1} for 3 days) induced amiloride-sensitive electrogenic Na^+ transport, with the potency of aldosterone > dexamethasone > RU 28362.

4. With each corticosteroid, increased electrogenic Na^+ transport reflected enhanced apical Na^+ conductance, and in the case of aldosterone and dexamethasone, 3·3-fold and 2-fold increases respectively in the maximum activity of the basolateral Na^+–K^+ pump. In contrast, RU 28362 suppressed the maximum activity of the basolateral Na^+–K^+ pump by 45%.

5. All three corticosteroids enhanced the K^+ conductance of the apical membrane, with the potency of aldosterone > dexamethasone > RU 28362.

6. Co-administration of spironolactone (5 mg 100 g^{-1} day^{-1}) inhibited the effects of aldosterone on Na^+ and K^+ transport, but in dexamethasone-treated animals spironolactone resulted in a pattern of response similar to that found in RU 28362-treated animals.

7. The results support the view that mineralocorticoid receptors mediate changes in colonic Na^+ and K^+ transport which differ quantitatively and qualitatively from those mediated by glucocorticoid receptors. Dexamethasone and similar 'glucocorticoids' activate both types of receptor, with an overall epithelial response which mimics that induced by aldosterone.

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INTRODUCTION

Mammalian colonic epithelia are prime targets for the action of corticosteroid hormones. The initial epithelial response is rapid, and stimulation of Na^+ and K^+ transport is evident in distal colon of rabbit, rat and man 1–5 h after *in vivo* or *in vitro* exposure to mineralocorticoid or glucocorticoid hormones (Binder, 1978; Frizzell & Schultz, 1978; Sandle, Hayslett & Binder, 1986; Sandle & McGlone, 1987*a*; Bridges, Rummel & Schreiner, 1987). Specific receptors for corticosteroid hormones are present in the cytosol of colonic epithelial cells (Bastl, Barnett, Schmidt & Litwack, 1984; Binder, White, Whiting & Hayslett, 1986), and changes in Na^+ and K^+ transport are the end-result of a sequence of events which involves the formation of an 'activated' hormone–receptor complex, movement of the complex to intranuclear binding sites, and the induction of transport proteins which ultimately appear in the cell membrane (Edelman, 1981; Schmidt & Litwack, 1982). While *in vivo* studies indicate that mineralocorticoids and glucocorticoids induce broadly similar changes in distal colonic Na^+ and K^+ transport (Charney, Kinsey, Myers, Giannella & Gots, 1975; Charney, Wallach, Ceccarelli, Donowitz & Costenbader, 1981), *in vitro* studies have exposed important differences which suggest that the two types of corticosteroid act via different populations of corticosteroid receptor (Foster, Zimmerman, Hayslett & Binder, 1983; Jorkasky, Cox & Feldman, 1985). Hyperaldosteronism, whether induced by dietary Na^+ depletion or by aldosterone infusion, activates specific mineralocorticoid receptors and produces a purely 'mineralocorticoid' pattern of response in colonic electrolyte transport (Foster *et al.* 1983; Halevy, Budinger, Hayslett & Binder, 1986). Most studies with glucocorticoid hormones have involved the administration of pharmacological doses, which results in appreciable cross-over binding to mineralocorticoid receptors as well as glucocorticoid receptor activation (Marusic, Hayslett & Binder, 1981; Binder *et al.* 1986; Bastl, 1987). It has therefore not been entirely clear whether the transport effects of these glucocorticoids mainly reflect (i) activation of specific glucocorticoid receptors, (ii) cross-over binding to mineralocorticoid receptors, or (iii) a combination of both these possibilities. The electrophysiological experiments described below were designed to establish and compare the effects of aldosterone, dexamethasone, and the glucocorticoid agonist RU 28362, on the Na^+ and K^+ transport properties of rat distal colonic epithelium. RU 28362 belongs to a class of synthetic glucocorticoids derived from 17α-alkynyl-11β,17dihydroxyandrostane which are virtually specific for the glucocorticoid receptor (Teutsch, Costerousse, Deraedt, Benzoni, Fortin & Philibert, 1981; Beaumont & Fanestil, 1983).

METHODS

Experiments were performed in six groups of non-fasting male Sprague–Dawley rats weighing 250–300 g. Group 1 (control) animals were fed 20 g day^{-1} of normal rat chow. Group 2 animals were fed 20 g day^{-1} of a Na^+-free paste diet for 10–14 days to induce secondary hyperaldosteronism, as previously described (Schon, Silva & Hayslett, 1974). Although dietary Na^+ depletion stimulates the renin–angiotensin system in addition to aldosterone secretion, the effects of dietary Na^+ depletion on Na^+ transport in rat distal colon have been shown to be produced by aldosterone (Halevy *et al.* 1986); for convenience group 2 animals are referred to as 'aldosterone-treated'. Group 3 (dexamethasone-treated) animals were fed normal chow and injected intraperitoneally

with 600 μg 100 g^{-1} day^{-1} of dexamethasone for 3 days, as the effects of this regime on cation fluxes across rat distal colon have been established in previous studies (Foster *et al.* 1983). Group 4 (RU 28362-treated) animals were fed normal chow and injected intraperitoneally with a similar dose (600 μg 100 g^{-1} day^{-1}) of RU 28362 (a gift of Roussel-UCLAF, Romainville, France) for 3 days. Group 5 (aldosterone–spironolactone-treated) animals were fed the Na^+-free diet for 10–14 days, and treated concurrently with 5 mg 100 g^{-1} of the mineralocorticoid receptor antagonist spironolactone (dissolved in 75% dimethylsulphoxide–25% sterile water, v/v) by daily intraperitoneal injection. Group 6 (dexamethasone–spironolactone-treated) animals were injected daily for 3 days with 600 μg 100 g^{-1} day^{-1} of dexamethasone and 5 mg 100 g^{-1} day^{-1} of spironolactone. A spironolactone-treated control group was not used as spironolactone (14 mg 100 g^{-1} day^{-1}) has no effect on basal colonic electrolyte transport in these animals (Charney *et al.* 1981). All animals were given tap water to drink *ad libitum*.

Animals were killed by cervical dislocation after stunning. The colon was removed, rinsed with NaCl Ringer solution (see below) at 37 °C, and stripped of serosa and underlying muscle layers. A 2 cm piece of distal colon (that segment beginning 4 cm from the anus) supported by a nylon mesh was mounted in a modified Ussing-type chamber (volume of each half-chamber 12 ml). Tissue area was 1 cm^2. Basal electrical properties and the effects of channel blockers were determined with tissues bathed in a solution containing (mM): Na^+, 136·2; K^+, 7·0; Cl^-, 121; Ca^{2+}, 2·0; Mg^{2+}, 1·2; HCO_3^-, 25; $H_2PO_4^-$, 1·2; SO_4^{2-}, 1·2; and glucose 11·1. The solution was maintained at 37 °C, gassed with a 95% O_2/5% CO_2 mixture, and had a pH of 7·4. All measurements were made under open-circuit conditions.

Microelectrodes were prepared from fibre-filled glass capillary tubing (internal diameter 1·5 mm), filled with 0·5 M-KCl, and had tip diameters of <1 μm and tip resistance of 40–100 MΩ in NaCl Ringer solution. Transepithelial voltage (V_t) and basolateral membrane voltage (V_b) measured during cell impalements were monitored using a microcomputer (BBC Model B)-based data acquisition system. Changes in V_t (ΔV_t) and V_b (ΔV_b) on passing a rectangular current pulse (I = 120 μA cm^{-2}, duration 2·5 s) were corrected for the series resistance of the bathing solution (Wills, Lewis & Eaton, 1979), and the change in apical membrane voltage (ΔV_a) calculated as $\Delta V_a = \Delta V_t - \Delta V_b$. After 15–25 min, when V_t, total tissue conductance ($G_t = I/\Delta V_t$), and equivalent short-circuit current ($I_{sc} = V_t G_t$) were stable, four to six impalements were performed from the apical side of the tissue using a manually operated 3-D hydraulic microdrive (Narishige Scientific Instrument Laboratories, Tokyo, Model MO-103), and the rectangular current pulse applied at 5–10 s intervals. The ratio $\Delta V_a/\Delta V_b$ was assumed to be equal to the apical/basolateral membrane resistance ratio (α), which was calculated by the microcomputer taking into account the series resistance of the solution bathing either side of the tissue (Wills *et al.* 1979).

Cell impalements were accepted if (i) V_b reached a constant value immediately on entering the cell; (ii) V_b and α were stable throughout the impalement, which lasted 1–10 min; (iii) the microelectrode reading returned to baseline (set to V_t before the impalement) on leaving the cell; and (iv) microelectrode tip resistance was unchanged by the impalement.

Effects of the Na^+ channel blocker amiloride (final concentration 0·1 mM) and the K^+ channel blocker tetraethylammonium chloride (TEA; final concentration 30 mM) were determined by adding the drugs to the mucosal solution during continuous impalements.

In order to assess basolateral Na^+–K^+ pump activity, tissues were bathed in a high-K^+, Na^+-free solution containing (mM): K^+, 140; HCO_3^-, 25; Ca^{2+}, 10 (methane sulphonate); Mg^{2+}, 1·2; $H_2PO_4^-$, 1·2; $MeSO_3^-$, 20; gluconate, 113·8; and glucose, 11·1. Nystatin (Sigma Chemical Co., St Louis, MO, USA; final concentration 480 U ml^{-1}) was added to the mucosal solution to increase apical membrane permeability to monovalent ions; under these conditions V_t and I_{sc} were zero. With 0·1 mM-amiloride in the mucosal solution, aliquots of a stock sodium gluconate solution were added first to the serosal side (with no effect) and then to the mucosal side in increments of 10 mM, to a final concentration of 50 mM. Increases in mucosal (and thus intracellular) Na^+ rapidly increased V_t and I_{sc}, which reached steady values after 2–3 min. Pump kinetics were evaluated from the changes in I_{sc} at increasing mucosal Na^+ concentrations ([Na^+]) using an iterative least-squares curve-fitting routine to fit the data to a model of highly co-operative binding:

$$I_{sc} = \frac{I_{sc,max}}{1 + \left(\frac{K_{Na}}{[Na^+]}\right)^n},$$

where $I_{sc,max}$ = apparent maximum short-circuit current; K_{Na} = $[Na^+]$ at which the slope of I_{sc} on $[Na^+]$ is maximal; and n (Hill coefficient) = number of Na^+ ions binding to each Na^+ pump site (Hill, 1910; Nelson & Blaustein, 1980).

Average values of basal transepithelial and microelectrode measurements were calculated for each tissue before calculating the mean ± S.E.M. for each group of tissues. The effects of amiloride and TEA were measured during continuous impalements. Statistical comparisons between groups were performed using the Mann–Whitney U test, and the effects of amiloride and TEA analysed using Student's t test for paired data.

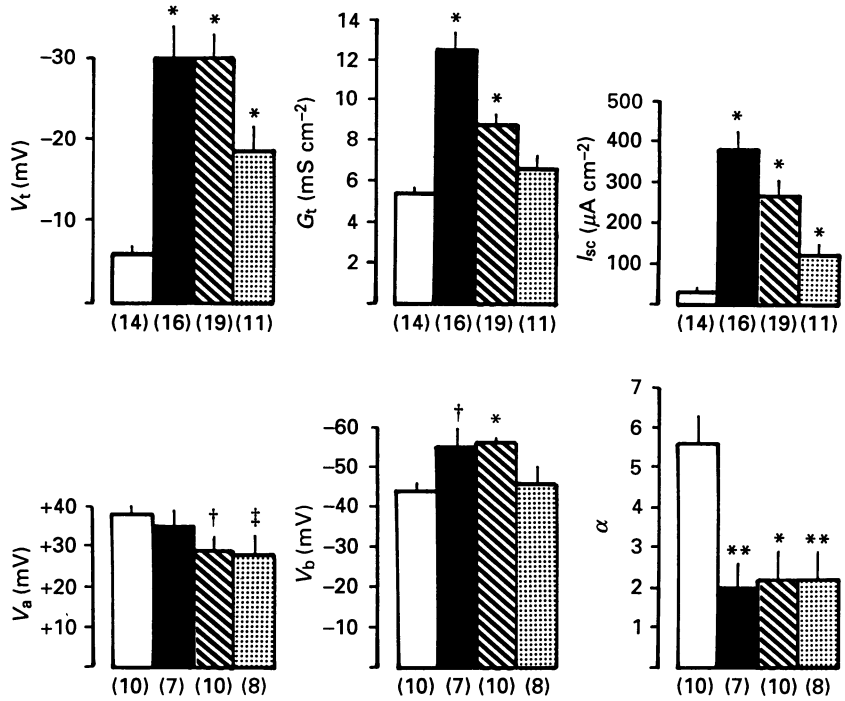

Fig. 1. Effects of aldosterone, dexamethasone and RU 28362 on the electrical properties of rat distal colon. Results are means ± S.E.M. in control (open bars), aldosterone-treated (filled bars), dexamethasone-treated (hatched bars) and RU 28362-treated (stippled bars) animals. V_t = transepithelial voltage (mucosal surface negative); G_t = total tissue conductance; I_{sc} = equivalent short-circuit current; V_a = apical membrane voltage (positive with respect to cell interior); V_b = basolateral membrane voltage (negative with respect to serosal solution); α = apical/basolateral membrane resistance ratio. *$P < 0.001$, **$P < 0.01$, †$P < 0.025$ and ‡$P < 0.05$ compared with controls. Numbers of tissues are shown in parentheses.

RESULTS

Effects of aldosterone, dexamethasone and RU 28362 on basal electrical properties

The effects of aldosterone, dexamethasone and RU 28362 on the basal electrical properties of rat distal colon are shown in Fig. 1. Compared with the control group, aldosterone stimulated a 5-fold increase in V_t ($P < 0.001$), which reflected 12-fold and 2·3-fold increases in I_{sc} ($P < 0.001$) and G_t ($P < 0.001$) respectively. Dexamethasone increased V_t 5-fold ($P < 0.001$) owing to 8·6-fold and 1·6-fold increases in I_{sc} ($P < 0.001$) and G_t ($P < 0.001$) respectively. Aldosterone and dexametha-

sone hyperpolarized the basolateral membrane by 11 mV ($P < 0.025$) and 12 mV ($P < 0.001$) respectively, and both corticosteroids decreased α by ~60%. It should be noted, however, that whereas aldosterone did not change V_a, dexamethasone depolarized the apical membrane by 9 mV ($P < 0.025$).

In contrast to aldosterone and dexamethasone, RU 28362 induced a modest 3-fold rise in V_t ($P < 0.001$) which mainly reflected the 4-fold increase in I_{sc} ($P < 0.001$), as G_t did not change. RU 28362, like dexamethasone, significantly decreased V_a by 9 mV ($P < 0.05$) and α by 61% ($P < 0.01$), but unlike dexamethasone, RU 28362 did not change V_b. Thus, some but not all of the electrical effects of RU 28362 were similar to those induced by dexamethasone.

These data indicate that all three corticosteroids induced changes consistent with electrogenic Na^+ transport, with the potency of aldosterone > dexamethasone > RU 28362. An interesting feature was the ability of the two glucocorticoid hormones, but not aldosterone, to depolarize the apical membrane (Fig. 1). Since corticosteroid-induced apical Na^+ and K^+ conductances would result in apical membrane depolarization and hyperpolarization respectively, the difference in V_a response suggests that aldosterone induced a greater apical K^+ conductance than either dexamethasone or RU 28362.

Effects of aldosterone, dexamethasone and RU 28362 on apical membrane conductance

Figure 2 (transepithelial data) and Fig. 3 (microelectrode data) summarize the effects of amiloride and TEA on rat distal colon. The channel blockers were used to assess the relative abilities of aldosterone, dexamethasone and RU 28362 to induce Na^+ and K^+ conductances in the apical membrane.

Apical Na^+ conductance

As previously reported (Sandle, Hayslett & Binder, 1984), amiloride had no effects in the control group (Figs 2 and 3), indicating that amiloride-sensitive apical Na^+ channels are normally absent from this epithelium. In the aldosterone-, dexamethasone- and RU 28362-treated groups, however, amiloride significantly decreased V_t, I_{sc} and G_t (Fig. 2) and significantly increased V_a and α without changing V_b (Fig. 3). These changes are consistent with the blockade by amiloride of apical Na^+ conducatances and the inhibition of varying degrees of electrogenic Na^+ transport induced by the three corticosteroids. The effects of amiloride on the transepithelial parameters (particularly I_{sc}) were most marked in the aldosterone-treated group, and least marked in the RU 28362-treated group. Figure 2 shows that I_{sc} in the aldosterone-treated group was completely abolished by amiloride, whereas the post-amiloride values of I_{sc} in the dexamethasone-treated group (54 ± 9 μA cm^{-2}) and the RU 28362-treated group (30 ± 5 μA cm^{-2}) were similar to the basal (31 ± 6 μA cm^{-2}) and post-amiloride (29 ± 6 μA cm^{-2}) values in the control group.

Apical K^+ conductance

In the control group, TEA significantly increased V_t and I_{sc} (Fig. 2), consistent with a decrease in K^+ movement from serosa to mucosa, but did not change V_a or α (Fig. 3). These results suggest that only a relatively small TEA-sensitive apical K^+ conductance is normally present in rat distal colon. In contrast, in the aldosterone-

and dexamethasone-treated groups, TEA not only increased V_t and I_{sc}, but also significantly decreased G_t and V_a, and increased α (Figs 2 and 3). Apart from the lack of change in G_t, TEA produced similar effects in the RU 28362-treated group as in the aldosterone- and dexamethasone-treated groups. It should be noted that before the addition of TEA, post-amiloride V_a in the aldosterone-treated group (54±5 mV) was

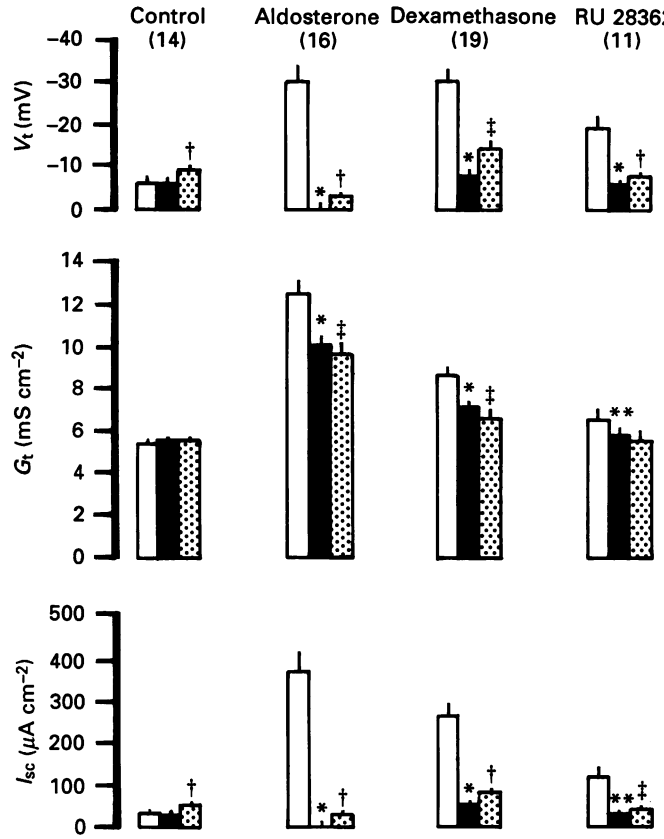

Fig. 2. Effects of amiloride (0·1 mM) and tetraethylammonium chloride (TEA, 30 mM) on transepithelial measurements in distal colon from control, aldosterone-, dexamethasone- and RU 28362-treated animals. Mean (±S.E.M.) values presented are those in the basal state (open bars), in the presence of amiloride (filled bars), and in the presence of amiloride plus TEA (stippled bars). V_t, G_t and I_{sc} are defined in legend to Fig. 1. *$P < 0·001$ and **$P < 0·01$ compared with basal or pre-amiloride value; †$P < 0·001$ and ‡$P < 0·01$ compared with value in the presence of amiloride. Numbers of tissues are shown in parentheses.

higher than in the control group (40±2 mV, $P < 0·02$), whereas post-amiloride V_a was similar in the control, dexamethasone- and RU 28362-treated groups (40±2, 46±4 and 40±4 mV respectively). The high post-amiloride V_a in the aldosterone-treated group suggests that aldosterone induced a greater apical K^+ conductance than either of the glucocorticoid hormones. Taken together, these results indicate that all three corticosteroid hormones induced apical K^+ conductances with the potency of aldosterone > dexamethasone > RU 28362.

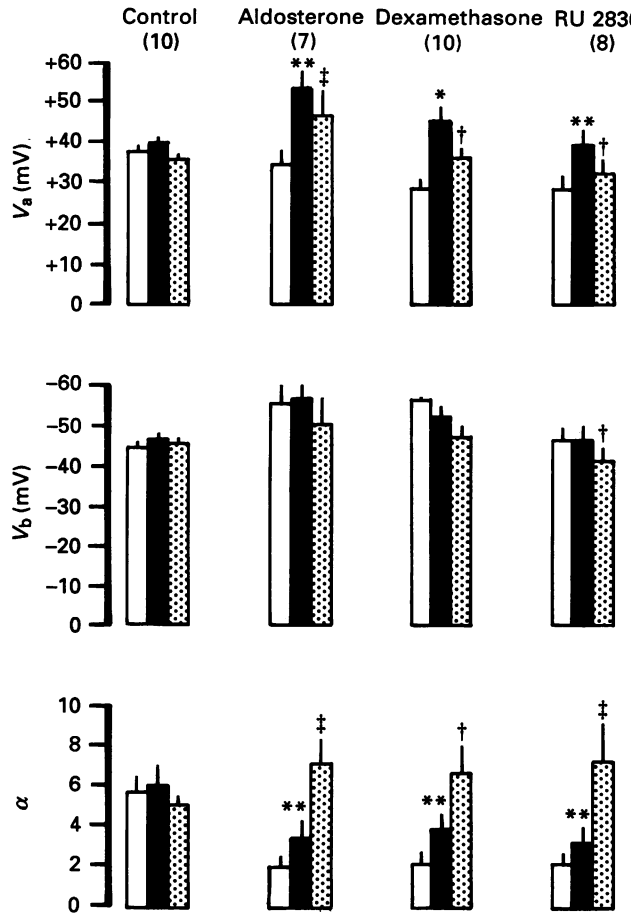

Fig. 3. Effects of amiloride (0·1 mM) and tetraethylammonium chloride (TEA, 30 mM) on microelectrode measurements in distal colon from control, aldosterone-, dexamethasone- and RU 28362-treated animals. Mean (±S.E.M.) values presented are those in the basal state (open bars), in the presence of amiloride (filled bars), and in the presence of amiloride plus TEA (stippled bars). V_a, V_b and α are defined in legend to Fig. 1. *$P < 0.001$ and **$P < 0.025$ compared with basal or pre-amiloride value; †$P < 0.025$ and ‡$P < 0.05$ compared with value in the presence of amiloride. Numbers of tissues are shown in parentheses.

Effects of aldosterone, dexamethasone and RU 28362 on the basolateral Na^+–K^+ pump

Figure 4 shows the responses of the I_{sc} in the control and corticosteroid-treated groups when the basolateral Na^+–K^+ pump was activated by stepwise increases in mucosal Na^+ concentration. Compared with the control group, I_{sc} was higher in the aldosterone- and dexamethasone-treated groups, but lower in the RU 28362-treated group, at each concentration of Na^+. The kinetic data derived from the best-fit curves (Table 1) indicate that compared with the control group, aldosterone and dexamethasone increased $I_{sc,max}$ 3·3-fold ($P < 0.001$) and 2-fold ($P < 0.01$) respectively, and values in the two corticosteroid-treated groups were significantly differ-

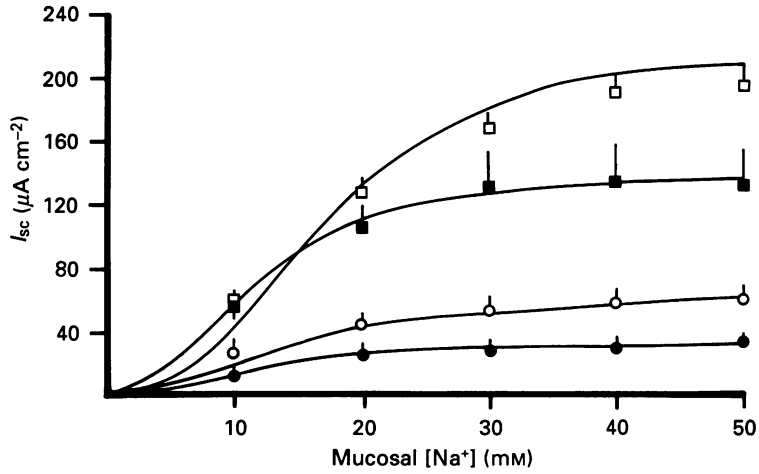

Fig. 4. Response of short-circuit current (I_{sc}) to increasing mucosal concentrations of Na^+ in distal colon from six control (○——○), seven aldosterone- (□——□), six dexamethasone- (■——■) and six RU 28362- (●——●) treated animals. Each point represents the mean (±S.E.M.) of the data at each concentration of Na^+ and the curves through the points are the best fits to the equation describing the model of highly co-operative binding (see Methods). Best-fit values for the lines are presented in Table 1.

TABLE 1. Kinetics of the basolateral Na^+–K^+ pump in distal colon from control, aldosterone-, dexamethasone- and RU 28362-treated animals

	$I_{sc,max}$ (μA cm^{-2})	K_{Na} (mM)	n
Control (6)	69±9	15±3	1·9±0·2
Aldosterone (7)	227±35	17±2	2·6±0·7
P	<0·001	n.s.	n.s.
Dexamethasone (6)	142±28	12±1	2·6±0·2
P	<0·01	n.s.	<0·05
RU 28362 (6)	38±6	12±2	1·7±0·2
P	<0·01	n.s.	n.s.

Results are expressed as means±S.E.M. $I_{sc,max}$ = the apparent maximum short-circuit current; K_{Na} = [Na^+] at which the slope of I_{sc} on [Na^+] is maximal; n (Hill coefficient) = the number of Na^+ ions binding to each Na^+–K^+ pump site. P = significance of difference from control. Numbers of tissues are shown in parentheses.

ent (227±35 *vs.* 142±28 μA cm^{-2}, $P < 0·05$). In marked contrast, $I_{sc,max}$ in the RU 28362-treated group was decreased by 45% ($P < 0·01$) below the control value. Apart from the relatively high Hill coefficient (n), in the dexamethasone-treated group there were no significant differences in the other Hill coefficients or the values of K_{Na} between the various groups.

Effects of aldosterone and dexamethasone in spironolactone-treated animals

Microelectrode measurements were not obtained in the spironolactone-treated animals, but in all other respects the experimental format was similar to that used in the other groups.

Figure 5 shows the transepithelial measurements in the control group and the

aldosterone- and dexamethasone-treated groups treated concurrently with spironolactone. There was no significant difference in V_t or I_{sc} between the control and aldosterone–spironolactone groups, although G_t was significantly higher in the animals treated with aldosterone and spironolactone. This difference in G_t may reflect effects of chronic Na^+ depletion on the mucosa which were not mediated by

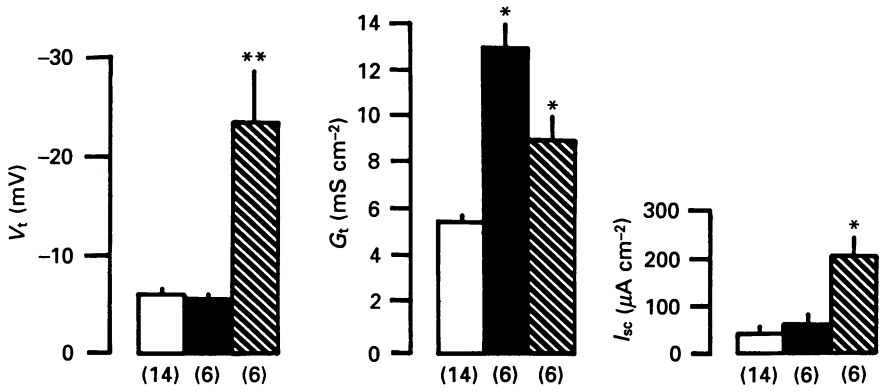

Fig. 5. Effects of aldosterone and dexamethasone on the electrical properties of rat distal colon in spironolactone-treated animals. Results are means ± S.E.M. in control (open bars), aldosterone–spironolactone-treated (filled bars) and dexamethasone–spironolactone-treated (hatched bars) animals. V_t, G_t and I_{sc} are defined in legend to Fig. 1. $*P < 0{\cdot}001$ and $**P < 0{\cdot}01$ compared with controls. Numbers of tissues are shown in parentheses.

aldosterone or angiotensin, as angiotensin stimulates electroneutral NaCl absorption in rat distal colon without changing the electrical properties of the mucosa (Munday & York, 1976). In contrast, dexamethasone–spironolactone treatment stimulated a 4-fold ($P < 0{\cdot}01$) increase in V_t, owing to 6·9-fold ($P < 0{\cdot}001$) and 1·7-fold ($P < 0{\cdot}001$) increases in I_{sc} and G_t respectively, and these changes were similar to those induced by RU 28362 and by dexamethasone alone (Fig. 1).

Figure 6 summarizes the effects of amiloride and TEA. As described earlier, amiloride and TEA had negligible effects in the control group. In the aldosterone–spironolactone-treated group, amiloride produced small but significant decreases in I_{sc} (43 μA cm^{-2}, $P < 0{\cdot}05$) and G_t (0·4 mS cm^{-2}, $P < 0{\cdot}05$) but TEA had no effect. Thus, spironolactone inhibited almost all of the stimulatory effect of aldosterone on electrogenic Na^+ transport, and completely prevented aldosterone from enhancing the K^+ conductance of the apical membrane. In contrast, in the dexamethasone–spironolactone-treated group, amiloride and TEA produced marked changes in V_t, I_{sc} and G_t consistent with the blockade of appreciable apical conductances to Na^+ and K^+ respectively. The effects of amiloride and TEA in the dexamethasone–spironolactone-treated group were generally less marked than those produced in the group treated with dexamethasone alone (cf. Fig. 2). Nevertheless, these results indicate that most of the stimulatory effects of dexamethasone on electrogenic Na^+ transport and the apical K^+ conductance were retained during treatment with spironolactone, and probably reflect binding of dexamethasone to specific (that is, spironolactone-insensitive) glucocorticoid receptors.

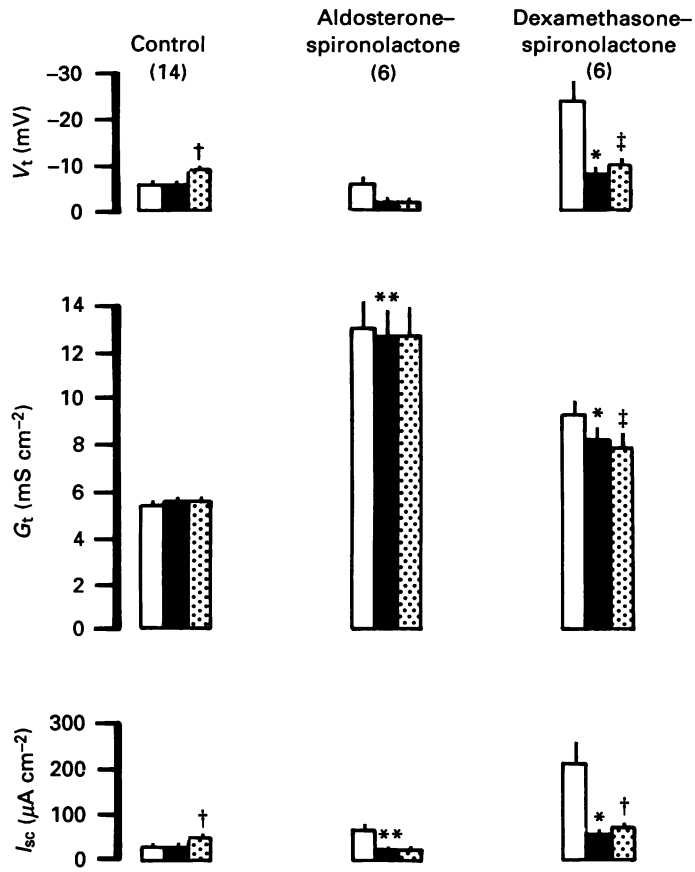

Fig. 6. Effects of amiloride (0·1 mM) and tetraethylammonium chloride (TEA, 30 mM) on transepithelial measurements in distal colon from control, aldosterone–spironolactone-treated, and dexamethasone–spironolactone-treated animals. Mean (±S.E.M.) values presented are those in the basal state (open bars), in the presence of amiloride (filled bars), and in the presence of amiloride plus TEA (stippled bars). V_t, G_t and I_{sc} are defined in legend to Fig. 1. *$P < 0·025$ and **$P < 0·05$ compared with basal or pre-amiloride value; †$P < 0·01$ and ‡$P < 0·025$ compared with value in the presence of amiloride. Numbers of tissues are shown in parentheses.

Figure 7 illustrates the changes in I_{sc} in the control, aldosterone–spironolactone-treated, and dexamethasone–spironolactone-treated groups when the basolateral Na^+–K^+ pump was activated by the mucosal addition of Na^+. In contrast to the group treated with aldosterone alone (Fig. 4), I_{sc} was similar in the control and aldosterone–spironolactone-treated groups at each Na^+ concentration and there were no differences in the kinetics of the pump between the two groups (Table 2). However, the response of the I_{sc} in the dexamethasone–spironolactone-treated group was similar to that obtained in the RU 28362-treated group (cf. Fig. 4); that is, the $I_{sc,max}$ was 30% ($P < 0·05$) lower in the dexamethasone–spironolactone-treated group than in the control group, with no significant changes in K_{Na} or the Hill coefficient.

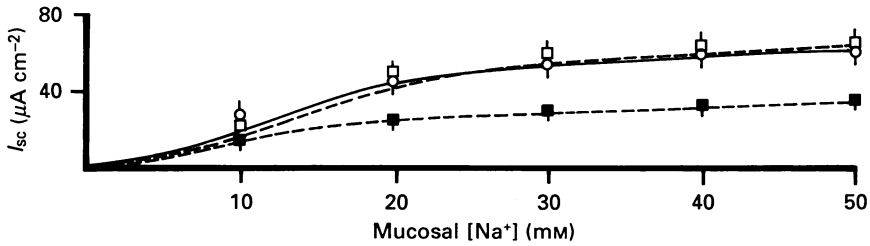

Fig. 7. Response of short-circuit current (I_{sc}) to increasing mucosal concentrations of Na^+ in distal colon from six control (○——○), six aldosterone–spironolactone-treated (□----□), and six dexamethasone–spironolactone-treated (■----■) animals. The data points and best-fit curves are as described in the legend to Fig. 4. Best-fit values for the lines are presented in Table 2.

TABLE 2. Kinetics of the basolateral Na^+–K^+ pump in distal colon from control, aldosterone–spironolactone-treated and dexamethasone–spironolactone-treated animals

	$I_{sc,max}$ (μA cm^{-2})	K_{Na} (mM)	n
Control (6)	69±9	15±3	1·9±0·2
Aldosterone–spironolactone (6)	77±11	20±4	1·5±0·2
P	n.s.	n.s.	n.s.
Dexamethasone–spironolactone (6)	48±7	19±3	1·1±0·3
P	<0·05	n.s.	n.s.

Results are expressed as means±S.E.M. $I_{sc,max}$, K_{Na} and n are defined in legend to Table 1. P = significance of difference from control. Numbers of tissues are shown in parentheses.

DISCUSSION

On the basis of previous studies, dietary Na^+ depletion resulted in maximal activation of mineralocorticoid receptors in rat distal colon. In adrenalectomized animals, aldosterone infusion produced maximal increases in distal colonic electrical potential difference when plasma aldosterone concentrations exceeded 0·75 ng ml^{-1}, and in adrenal-intact animals dietary Na^+ depletion increased plasma aldosterone concentrations to >4·5 ng ml^{-1} (Martin, Jones & Hayslett, 1983). It also seems likely that the high dose of RU 28362 resulted in almost full occupancy of glucocorticoid receptors (Bastl, 1987). Given these assumptions, it is clear that both aldosterone and RU 28362 enhanced apical membrane conductance to Na^+ and K^+, but aldosterone increased the maximum activity of the basolateral Na^+–K^+ pump by 230%, whereas RU 28362 decreased pump activity by 45%. Dexamethasone, when administered at doses judged to fully activate glucocorticoid receptors and result in substantial occupancy of mineralocorticoid receptors, induced changes in the Na^+ and K^+ transport properties of the apical and basolateral membranes which more closely resembled the 'mineralocorticoid' rather than the 'glucocorticoid' pattern of response. Concurrent administration of dexamethasone and spironolactone, however, resulted in changes in the cell membranes which mimicked those induced by the specific glucocorticoid RU 28362.

Implications for Na^+ transport

Recent studies in rat distal colon indicate that continuous infusions of aldosterone *in vivo* initially increase electroneutral NaCl absorption (normally the predominant Na^+ absorptive process); this effect is short-lived and is seen at 24 h but not at 48 h (Halevy *et al.* 1986). The principal effect of aldosterone, which is clearly established at 24 h and becomes more marked thereafter, is to enhance amiloride-sensitive electrogenic Na^+ transport (Halevy *et al.* 1986). Stimulation of rat distal colon by aldosterone for 3–7 days or longer further enhances amiloride-sensitive electrogenic Na^+ transport and completely inhibits amiloride-insensitive electroneutral NaCl absorption (Foster *et al.* 1983; Halevy *et al.* 1986). Experimental evidence from several sources suggests that the increases in basolateral membrane area and Na^+, K^+-ATPase content induced by chronic hyperaldosteronism (Kashgarian, Taylor, Binder & Hayslett, 1980) are secondary to a sustained increase in apical Na^+ entry rather than a direct effect of aldosterone on the basolateral membrane (Frizzell & Schultz, 1978; Will, DeLisle, Cortright & Hopfer, 1981; Halm & Dawson, 1985; Halevy, Boulpaep, Binder & Hayslett, 1985). Consequently, hyperpolarization of the basolateral membrane observed in the aldosterone-treated tissues may reflect a rise in intracellular K^+ concentration owing to increased basolateral K^+ uptake, as we have demonstrated that V_b, intracellular K^+ concentration, and basolateral Na^+,K^+ ATPase activity increase in parallel in two other models of secondary hyperaldosteronism, namely chronic 12-fold dietary K^+ enrichment in renal-intact animals, and chronic 4-fold dietary K^+ enrichment in partially nephrectomized animals (Sandle, Foster, Lewis, Binder & Hayslett, 1985; Sandle, McGlone & Davies, 1988). The combination of greatly increased electrogenic Na^+ transport secondary to enhanced apical Na^+ entry and basolateral Na^+–K^+ pump activity, together with total inhibition of electroneutral NaCl absorption, typifies the chronic effects of aldosterone on Na^+ transport in rat distal colon.

The present study appears to be the first to compare the effects of specific glucocorticoid and mineralocorticoid receptor activation in mammalian colon. Amiloride-sensitive I_{sc} and G_t were induced less effectively by RU 28362 than by aldosterone (I_{sc}: 91 ± 23 *vs.* 366 ± 56 μA cm^{-2}, $P < 0{\cdot}002$; and G_t: $0{\cdot}8 \pm 0{\cdot}2$ *vs.* $2{\cdot}5 \pm 0{\cdot}4$ mS cm^{-2}, $P < 0{\cdot}002$). Apical membrane hyperpolarization produced by amiloride-blockade of the apical Na^+ conductance was also less marked in the RU 28362-treated group than in the aldosterone-treated group (11 ± 4 *vs.* 19 ± 4 mV, Fig. 3), although this difference was not statistically significant. The level of electrogenic Na^+ transport produced by glucocorticoid receptor activation was therefore much lower than that produced by mineralocorticoid receptor activation, a difference which at least partly reflects opposite effects of RU 28362 and aldosterone on the maximum activity of the basolateral Na^+–K^+ pump. Because the specific glucocorticoid RU 26988 has been shown to increase distal colonic Na^+ absorption, K^+ secretion, and *in vivo* transmucosal potential difference in adrenalectomized animals (Bastl *et al.* 1984), suppression of Na^+–K^+ pump activity by RU 28362 was an unexpected finding. One explanation for this paradox may be that sufficient pump activity remained in the RU 28362-treated tissues to accommodate the increase in apical Na^+ entry, as studies in rabbit descending colon

indicate that the basolateral Na^+–K^+ pump normally operates at ~50% of its maximum capacity (Frizzell & Schultz, 1978). In the rat, plasma levels of corticosterone exceed those of aldosterone by several order of magnitude (Martin *et al.* 1983), and corticosterone binds to mineralocorticoid receptors as well as to glucocorticoid receptors (Marusic *et al.* 1981; Lan, Graham, Bartter & Baxter, 1982). Administration of RU 28362 may therefore have inhibited secretion of endogenous corticosterone, thus effectively removing the mineralocorticoid 'tone', leaving Na^+ transport solely under glucocorticoid control.

While more effective than RU 28362, dexamethasone induced a smaller amiloride-sensitive I_{sc} than aldosterone (212 ± 28 *vs.* 366 ± 56 $\mu A\ cm^{-2}$, $P < 0.05$). A similar difference has been noted in voltage-clamped rat distal colon, in which the amiloride-sensitive components of the net Na^+ absorptive flux and I_{sc} were 100% greater in Na^+-depleted than in dexamethasone-treated animals (Foster *et al.* 1983). In addition, electroneutral NaCl absorption was completely inhibited by aldosterone, whereas dexamethasone had no appreciable effect (Foster *et al.* 1983). Since dexamethasone administered at high dosage binds substantially to mineralocorticoid receptors (Marusic *et al.* 1981), spironolactone was used to determine whether the effects of dexamethasone reflected activation of glucocorticoid, mineralocorticoid or both types of receptor. We expected that if the effects of dexamethasone mainly reflected mineralocorticoid receptor activation, they would be largely inhibited by spironolactone, and the electrical properties of the epithelium would be similar to those in the aldosterone–spironolactone-treated group. However, co-administration of spironolactone and dexamethasone resulted in changes in the Na^+ transport properties of the apical and basolateral membranes which closely resembled those induced by RU 28362. Thus, when administered alone, the effects of dexamethasone on apical Na^+ conductance and basolateral Na^+–K^+ pump activity reflect a combination of mineralocorticoid and glucocorticoid receptor activation. It remains unclear why dexamethasone (unlike aldosterone) has no inhibitory effect on electroneutral NaCl absorption, although this difference may reflect different degrees of mineralocorticoid receptor occupancy, or the fact that distal colon was exposed to aldosterone for longer than dexamethasone.

Implications for K^+ transport

In normal rat distal colon, Na^+–K^+-ATPase-mediated basolateral K^+ uptake maintains intracellular K^+ above its equilibrium potential, and V_b is predominantly a K^+ diffusion potential across the highly K^+-conductive basolateral membrane (Sandle *et al.* 1985). The apical membrane has a negligible K^+ conductance (Sandle *et al.* 1985; Sandle & McGlone, 1987*b*), and also contains an electroneutral K^+ uptake mechanism which may be a K^+–H^+ exchange, as it is Na^+-independent and only partially Cl^--dependent (Foster, Hayslett & Binder, 1984). The unidirectional K^+ flux from mucosa to serosa (J^K_{ms}) therefore exceeds the unidirectional K^+ flux from serosa to mucosa (J^K_{sm}), resulting in net K^+ absorption (Foster *et al.* 1984). Previous studies *in vivo* and *in vitro* have established that chronic mineralocorticoid excess enhances the K^+ secretory capacity of rat distal colon by stimulating an active (transcellular) K^+ secretory process, and by increasing passive (voltage-dependent) K^+ movement into the lumen via paracellular pathways (Edmonds, 1981; Charney

et al. 1981; Foster *et al.* 1983; Foster *et al.* 1984). Studies designed to investigate the effects of specific glucocorticoid hormones (i.e. RU 26988) have been performed *in vivo* in adrenalectomized animals, and as RU 26988 increased transmucosal voltage, the associated increase in K^+ secretion may reflect passive K^+ movement (Bastl *et al.* 1984). In the present study in adrenal-intact animals the specific glucocorticoid RU 28362 increased the lumen-negative transepithelial voltage, and inhibited basolateral Na^+–K^+ pump activity. It therefore seems likely that under conditions *in vivo*, K^+ secretion induced by specific glucocorticoid hormones reflects predominantly voltage-dependent K^+ diffusion via paracellular pathways and across the K^+-conductive apical membrane. In contrast, chronic hyperaldosteronism and chronic dexamethasone administration stimulate active K^+ secretory processes in addition to enhancing voltage-dependent K^+ transport, and furthermore, marked qualitative and quantitive differences exist between the active K^+ secretory processes induced by the two corticosteroids (Foster *et al.* 1983, 1984). Under voltage-clamp conditions, aldosterone enhances J^K_{sm} and decreases J^K_{ms}, thereby reversing net K^+ absorption to net K^+ secretion; dexamethasone increases J^K_{sm} but has no effect on J^K_{ms}, and decreases net K^+ transport to zero (Foster *et al.* 1983). The increases in J^K_{sm} induced by both aldosterone and dexamethasone appear to represent enhanced K^+ uptake at the basolateral membrane, a view supported by previous studies in which chronic hyperaldosteronism amplified both the area and Na^+,K^+-ATPase content of the basolateral membrane (by 45 and 78% respectively), while chronic dexamethasone administration induced similar changes (Kashgarian *et al.* 1980). In keeping with these findings, the present study indicates that aldosterone and dexamethasone enhanced the maximum activity of the basolateral Na^+–K^+ pump, and hyperpolarized the basolateral membrane (which may chiefly reflect a rise in intracellular K^+). In addition, both corticosteroids induced appreciable K^+ conductances in the apical membrane. Thus, the ability of aldosterone and dexamethasone to increase J^K_{sm} reflects the synergistic effects of enhanced basolateral K^+ uptake and increased apical K^+ exit along favourable electrochemical gradients.

Two features of the present study suggest that aldosterone induced a larger apical K^+ conductance than dexamethasone. First, despite the fact that aldosterone stimulated more electrogenic Na^+ transport than dexamethasone, V_a was similar in the aldosterone-treated and control groups ($+35 \pm 4$ and $+38 \pm 2$ mV respectively, Fig. 1), whereas V_a was lower in the dexamethasone-treated group than in the control group ($+29 \pm 3$ *vs.* 38 ± 2 mV, $P < 0{\cdot}025$, Fig. 1). Aldosterone may therefore have induced an exceptionally large apical K^+ conductance which offset the depolarizing effect of the parallel apical Na^+ conductance. Second, as shown in Fig. 3, the post-amiloride value of V_a was greater in the aldosterone-treated group than in the control group ($+54 \pm 5$ *vs.* $+40 \pm 2$ mV, $P < 0{\cdot}02$), while post-amiloride values of V_a were similar in the dexamethasone-treated and control groups ($+46 \pm 4$ and $+40 \pm 2$ mV respectively). Thus, with the apical Na^+ conductance inhibited by amiloride, the difference in V_a between the aldosterone-treated and control groups (~ 14 mV) reflected the aldosterone-induced apical K^+ conductance. This finding is of particular interest, as the results of several studies suggest that this K^+ conductance is large enough to produce recycling of K^+ across the apical membrane (Binder, Foster &

Hayslett, 1985), and it is this which accounts for the decrease in J^{K}_{ms} in aldosterone-treated distal colon, viz: (i) bathed in Na^+-free solutions, aldosterone-treated distal colon exhibits marked net K^+ absorption, indicating that aldosterone has no direct inhibitory effect on the apical K^+ absorptive process (Foster *et al.* 1984); (ii) mucosal addition of TEA to aldosterone-treated tissues bathed in Na^+-containing solutions reverses net K^+ secretion to net K^+ absorption by increasing J^{K}_{ms} as well as by decreasing J^{K}_{sm} (Binder & Sweiry, 1988); and (iii) mucosal TEA produces electrical changes in aldosterone-treated tissues consistent with the blockade of apical K^+ channels (Figs 2 and 3).

Some comment is required concerning the increase in apical Na^+ conductance induced by RU 28362, as the results of the present and previous studies (Bastl *et al.* 1984; Bastl, 1987) with specific glucocorticoid receptor agonists might be regarded as conflicting. Although RU 28362 and related compounds do not bind to mineralocorticoid receptors, we used a dose of RU 28362 far in excess of the doses of RU 26988 used in previous studies. The high dose of RU 28362 (600 μg 100 g^{-1} day^{-1}) allowed a comparison with the electrophysiological data and previous studies of ion transport which employed an identical dose of dexamethasone. Based on results in adrenalectomized animals, Bastl (1987) concluded that RU 26988 enhanced electroneutral but not electrogenic Na^+ absorption in rat distal colon, and that stimulation of electrogenic Na^+ transport by 'traditional' glucocorticoids (i.e. dexamethasone, methylprednisolone) reflected cross-over binding to mineralocorticoid receptors. However, in those studies, relatively low doses of RU 26988 (10–120 nmol 100 g^{-1} day^{-1} $\simeq$ 3–36 μg 100 g^{-1} day^{-1}) also increased the transmural potential difference in distal colon to normal levels. This response indicates that the glucocorticoid receptor may alter the electrical properties of the apical membrane in addition to enhancing electroneutral Na^+ absorption. Thus, in the present study, the increases in apical Na^+ conductance and I_{sc} induced by RU 28362 may represent an inherent property of glucocorticoid receptors which was activated by high doses of the glucocorticoid, and not cross-over binding to mineralocorticoid receptors.

In summary, the results of this study support the view that the effects of specific mineralocorticoid and glucocorticoid hormones on colonic Na^+ and K^+ transport are mediated by separate and distinct types of mineralocorticoid and glucocorticoid receptor. Changes in colonic cation transport elicited by dexamethasone, and probably by other synthetic 'glucocorticoids', reflect a combination of glucocorticoid receptor activation and, perhaps more importantly, substantial cross-over binding to mineralocorticoid receptors.

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