Segmental variability of glucocorticoid induced electrolyte transport in rat colon

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Abstract

Recent studies suggest that the ability of glucocorticoids to reduce diarrhoea in active colitis may reflect their direct effects on distal colonic electrogenic Na^+ transport and water absorption, as well as their anti-inflammatory action. To determine whether glucocorticoids induce similar changes in proximal colon, specific Na^+ and K^+ channel blockers (amiloride and tetraethylammonium chloride (TEA) respectively) were used to evaluate the cation transport properties of rat proximal and distal colon in vitro after three days treatment with the glucocorticoid agonist dexamethasone (600 $\mu\text{g}/100$ g/day). In the proximal colon, dexamethasone increased short circuit current (I_{sc}) 2.3 fold ($p < 0.025$) and total conductance (G_t) by 87% ($p < 0.015$), but had negligible effects on the maximum activity of the basolateral membrane Na^+/K^+ pump and the baseline Na^+ and K^+ conductive properties of the apical membrane. Additional studies with diphenylamine-2-carboxylic acid (a Cl^- channel blocker) suggested that the dexamethasone induced increases in I_{sc} and G_t in proximal colon reflected stimulation of an electrogenic Cl^- secretory process. In contrast, in the distal colon dexamethasone increased I_{sc} 10 fold ($p < 0.025$), G_t by 100% ($p < 0.015$), and the maximum activity of the basolateral Na^+/K^+ pump by 200% ($p < 0.05$), and induced substantial Na^+ and K^+ conductances in the apical membrane. These results indicate that dexamethasone stimulates electrogenic Na^+ transport and water absorption to a significant degree only in the distal segment of rat colon. Thus in patients with active colitis, that part of the antidiarrhoeal action of glucocorticoids that reflects stimulation of electrogenic Na^+ transport (and hence water absorption) may be restricted to the descending colon and rectum.

Oral or intravenous glucocorticoid agonists form the cornerstone of treatment in patients with acute inflammatory disease of the large bowel.¹⁻³ Until recently the efficacy of glucocorticoids was assumed to reflect their well known anti-inflammatory effects but it is now clear that pharmacological doses of glucocorticoids administered parenterally enhance rectal sodium (Na^+) and water absorption to the same extent in patients with active ulcerative colitis and in normal subjects.⁴ It is also apparent that the predominant mechanism for Na^+ absorption in human distal colon and rectum is electrogenic,^{5,6} and stimulated by both mineralocorticoid and glucocorticoid agonists.^{4,7} These observations suggest that glucocorticoids (which at high doses bind substantially to mineralocorticoid receptors

within colonic mucosa⁸), reduce diarrhoea in active colitis by stimulating distal colonic Na^+ and water absorption as well as by suppressing the underlying inflammatory process. It remains unclear, however, whether glucocorticoids also stimulate electrogenic Na^+ and water absorption in human proximal colon.

The assessment of glucocorticoid induced electrolyte transport in different segments of human colon in vivo poses a number of technical problems and would provide little information about changes in the biophysical properties of the colonic mucosa. The present study was therefore designed to determine the effects of long term dexamethasone treatment on the cell membrane barriers and forces controlling Na^+ and K^+ transport in rat proximal and distal colon. The results indicate that pharmacological doses of this glucocorticoid agonist induce changes in the properties of epithelial cell membranes consistent with the stimulation of electrogenic Na^+ absorption and K^+ secretion in the distal but not in the proximal colonic segment.

Methods

Experiments were performed in non-fasting male Sprague-Dawley rats weighting 250-300 g. Control animals were fed 20 g day of normal rat chow containing 2.8 mmol Na^+ . Glucocorticoid treated animals were fed normal rat chow and injected intraperitoneally with 600 $\mu\text{g}/100$ g/day of dexamethasone for three days. Dexamethasone was used because its effects on the ion transport and electrical properties of rat distal colon have previously been studied in detail.^{9,10} All animals were allowed tap water to drink *ad libitum*. Animals were killed by cervical dislocation, those in the glucocorticoid treated group being killed 24 hours after the final injection of dexamethasone. The colon was rinsed with NaCl Ringer solution containing (in mmol/l): 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 and was maintained at 37°C and pH 7.4 by gassing with a 95% O_2 :5% CO_2 mixture.

The colon was stripped of serosa and muscle layers, and a 2 cm piece of distal colon (that segment beginning 4 cm from the anus) or proximal colon (that segment immediately adjacent to the caecum) was mounted in an Ussing type chamber modified to eliminate tissue edge damage.¹¹ Tissue area was 1 cm^2 . Tissues were bathed on both sides with 12 ml NaCl Ringer solution and were temperature and pH regulated as described above. Electrical measurements were obtained under open circuit conditions. Transepithelial voltage (V_t) was monitored continuously with salt bridges (4% agar in 0.5 mol/l KCl) placed on either side of the tissue and

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connected to a voltmeter via balanced calomel half cells. Using Ohm's law, total tissue conductance (G_t) was calculated from the increase in V_t that occurred in response to a rectangular current pulse ($120 \mu\text{A}/\text{cm}^2$, 2.5 seconds duration) applied across the mucosa via an Anapulse stimulator (WPI, New Haven, CT, USA). The equivalent short circuit current (I_{sc}) was calculated as $I_{sc} = V_t \cdot G_t$.

Baseline electrical measurements were obtained after 20–25 minutes when V_t and G_t were stable. Effects of the Na^+ channel blocker amiloride (final concentration 0.1 mmol/l) and the K^+ channel blocker tetraethylammonium chloride (TEA; final concentration 30 mmol/l) were determined by adding the drugs sequentially to the mucosal solution. Electrical measurements were made five minutes after the amiloride and TEA (in the presence of amiloride) had been added when V_t and G_t were stable.

Dexamethasone treatment stimulated an amiloride insensitive increase in I_{sc} in the proximal colon (see Results) which suggested that the glucocorticoid induced an electrogenic anion secretory process in this segment. Similar changes were not observed, however, in the distal colon. To study this further, tissues were bathed in NaCl Ringer solution and 0.1 mmol/l amiloride was added to the mucosal solution to inhibit any component of I_{sc} which may have reflected electrogenic Na^+ transport. The Cl^- conductive properties of the apical membrane were then evaluated by measuring changes in I_{sc} produced by adding the Cl^- channel blocker diphenylamine-2-carboxylic acid (final concentration 2.5 mmol/l) to the mucosal solution.

Basolateral Na^+/K^+ pump activity was assessed as described previously.¹² Tissues were bathed on both sides in a high K^+ , Na^+ and Cl^- free solution containing (in mmol/l): 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 was added to the mucosal solution (final concentration 960 U/ml) to render the apical membrane freely permeable to monovalent ions,¹³ and under these conditions V_t and I_{sc} were zero. Amiloride (0.1 mmol/l) was added to the mucosal solution to block native Na^+ channels, and the Na^+ concentration of the serosal and then the mucosal solution raised in 10 mmol/l increments (using a stock Na^+ gluconate solution), to a final concentration of 50 mmol/l . Serosal addition of Na^+ had no effect, but increasing the mucosal (and intracellular) Na^+ concentration increased V_t and I_{sc} to stable values after two to

three minutes. Pump activity was evaluated from the increases in I_{sc} measured at increasing mucosal Na^+ concentrations, using an iterative least squares routine to fit the data to a mathematical model describing the highly cooperative binding of Na^+ to Na^+/K^+ pump sites.¹⁴ This model yields several kinetic parameters: $I_{sc,\text{max}}$ = apparent maximum short circuit current, K_{Na} = Na^+ concentration at which the slope of I_{sc} on Na^+ concentration is maximal, and n (Hill coefficient) = the minimum number of Na^+ ions binding to each Na^+/K^+ pump site.

Student's t test (two tailed) was used to make statistical comparisons between groups (non-paired test), and to assess the significance of the changes induced by amiloride and TEA (paired test).

Results

EFFECTS OF DEXAMETHASONE ON BASELINE ELECTRICAL PROPERTIES

The basal electrical properties of proximal and distal colon from control and dexamethasone treated animals are summarised in Table I.

In control animals, I_{sc} was similar in the proximal and distal colon, but V_t was significantly greater in the distal colon owing to the lower electrical conductance (G_t) in this segment. In the proximal colon, dexamethasone increased I_{sc} and G_t by 129% and 87% respectively above control values, while V_t was unchanged. In contrast, in the distal colon dexamethasone increased I_{sc} 10 fold and G_t by 100%, resulting in a 4.5 fold increase in V_t above the control value. Thus, while dexamethasone increased G_t in the proximal and distal colon, stimulation of I_{sc} (and V_t) was far more pronounced in the distal segment.

EFFECTS OF AMILORIDE AND TEA

The effects of amiloride and TEA in proximal and distal colon from control and dexamethasone treated animals are shown in Figure 1.

In proximal colon from control animals, the mucosal addition of amiloride and TEA had no effect on V_t , G_t or I_{sc} , indicating that apical Na^+ and K^+ conductances were negligible or absent.

In proximal colon from dexamethasone treated animals, in which baseline G_t was higher than in controls (Table I), amiloride and TEA had no effect on V_t , G_t or I_{sc} (Fig 1), which suggests that the dexamethasone stimulated rise in baseline G_t in this segment reflected the induction of a conductive pathway for ions other than Na^+ and K^+ .

In distal colon from control animals, amiloride produced small decreases in V_t (2.6 mV , $p < 0.05$) but no change in G_t or I_{sc} , and TEA was without effect. These results indicate that in this group of tissues, apical cation conductances were relatively small (in the case of Na^+) or absent (in the case of K^+).

In contrast to the other three groups, amiloride and TEA produced noticeable changes in the electrical properties of distal colon from dexamethasone treated animals. The addition of

TABLE I Baseline electrical measurements in proximal and distal colon from control and dexamethasone treated animals (values mean (SEM))

	V_t (mV)	G_t (mS/cm ²)	I_{sc} ($\mu\text{A}/\text{cm}^2$)
Proximal colon:			
Control rats (n=10)	-2.5 (0.4)	19.3 (3.4)	45 (12)
Dexamethasone treated rats (n=10)	-3.4 (0.7)	36.1 (5.8)	103 (18)
p**	NS	<0.015	<0.025
Distal colon:			
Control rats (n=12)	-7.3 (0.8)*	8.8 (0.8)*	62 (8)
Dexamethasone treated rats (n=9)	-33.0 (1.3)	17.7 (2.6)	618 (130)
p**	<0.01	<0.015	<0.0025

V_t = transepithelial voltage (negative with respect to serosal solution); G_t = total conductance;

I_{sc} = calculated short circuit current; n = number of tissues studied.

* = <0.05 compared with proximal colon from control animals.

** = difference between control and dexamethasone treated tissues.

Figure 1: Effects of amiloride (0.1 mmol/l) and tetraethylammonium chloride (TEA, 30 mmol/l) on transepithelial measurements in proximal and distal colon from control and dexamethasone treated animals. Mean (SEM) values presented are those under baseline conditions (open bars), in the presence of amiloride (filled bars), and in the presence of amiloride plus TEA (stippled bars). V_t , G_i , and I_{sc} are defined in footnote to Table I. * $p < 0.05$ compared with basal or pre-amiloride value; ** $p < 0.05$ compared with value in the presence of amiloride alone (Bonferroni correction applied to p values). n = number of tissues studied.

amiloride decreased V_t by 27.3 mV ($p < 0.05$), which reflected appreciable decreases in I_{sc} (561 $\mu\text{A}/\text{cm}^2$, $p < 0.05$) and G_i (6.5 mS/cm^2 , $p < 0.05$). These changes indicate that amiloride blocked a substantial apical Na^+ conductive pathway, and thereby inhibited the electrogenic Na^+ transport process induced by dexamethasone. The subsequent addition of TEA increased I_{sc} (by 37 $\mu\text{A}/\text{cm}^2$, $p < 0.025$), and V_t (by 4.4 mV, $p < 0.025$), changes consistent with the blockade of a dexamethasone induced apical K^+ conductive pathway.

TABLE II Effects of diphenylamine-2-carboxylic acid (DPC) on amiloride insensitive calculated short circuit current (I_{sc}) in proximal and distal colon from control and dexamethasone treated animals (values mean (SEM))

	$I_{sc}(\mu\text{A}/\text{cm}^2)$ pre-DPC	$I_{sc}(\mu\text{A}/\text{cm}^2)$ post-DPC	p
Proximal colon:			
Control rats ($n=4$)	74 (12)	9 (7)	<0.05
Dexamethasone treated rats ($n=7$)	139 (16)	3 (2)	<0.0001
Distal colon:			
Control rats ($n=4$)	22 (3)	6 (2)	<0.025
Dexamethasone treated rats ($n=5$)	30 (6)	14 (1)	0.06

n = number of tissues studied. Steady state measurements of I_{sc} were obtained with tissues bathed in NaCl Ringer solution (in the presence of 0.1 mmol/l mucosal amiloride) before and after the addition of 2.5 mmol/l DPC to the mucosal side.

EFFECTS OF DIPHENYLAMINE-2-CARBOXYLIC ACID (DPC)

Bathed in NaCl Ringer solution, proximal and distal colonic segments from control animals generally show a variable but modest I_{sc} which may reflect, at least in part, the Cl^- secretory tone of the tissues, as I_{sc} decreases when Cl^- is replaced with gluconate.¹⁵ Dexamethasone produced an amiloride and TEA insensitive increase in I_{sc} in the proximal colon (Table I and Fig 1), consistent with the stimulation of an electrogenic Cl^- secretory process. The effects of the Cl^- channel blocker DPC on the amiloride insensitive component of I_{sc} were therefore studied in proximal and distal colon from control and dexamethasone treated animals, and the results are summarised in Table II.

In distal colon from control and dexamethasone treated animals, and proximal colon from control animals, DPC produced relatively modest decreases in amiloride insensitive I_{sc} , consistent with the blockade of a small native apical Cl^- conductance in each of these three groups. In contrast, the high amiloride insensitive I_{sc} in proximal colon from dexamethasone treated animals (mean 139 (SEM) (16) $\mu\text{A}/\text{cm}^2$) was abolished by DPC, which suggests that dexamethasone induced a substantial apical Cl^- conductance in the proximal segment.

EFFECTS OF DEXAMETHASONE ON THE BASOLATERAL Na^+/K^+ PUMP

Figure 2 shows the changes in I_{sc} that occurred in response to 10 mmol/l increases in the mucosal Na^+ concentration in colonic segments from control and dexamethasone treated animals. The kinetic parameters derived from the best fit curves (Fig 2) are summarised in Table III. In the proximal colon, I_{sc} at each concentration of Na^+ tended to be greater in the dexamethasone treated than in the control animals, although these differences were not significant, and $I_{sc, \text{max}}$, K_{Na} , and n were similar in the two groups. In the distal colon, however, I_{sc} at each concentration of Na^+ was two to three times greater in the dexamethasone treated than in the control animals ($p < 0.05$), and $I_{sc, \text{max}}$ was increased

Figure 2: Response of short circuit current (I_{sc}) to increasing mucosal concentrations of Na^+ in control ($\circ$ — $\circ$, $n=5$) and dexamethasone-treated ($\bullet$ — $\bullet$, $n=7$) proximal colon, and control ($\square$ — $\square$, $n=6$) and dexamethasone-treated ($\blacksquare$ — $\blacksquare$, $n=8$) distal colon. Each point represents the mean (SEM) of the data at each concentration of Na^+ and the curves are best fits as described by the model of highly cooperative binding (see Methods). Best fit values for the curves are presented in Table III.

TABLE III Kinetics of the basolateral Na⁺-K⁺ pump in proximal and distal colon from control and dexamethasone treated animals (values mean (SEM))

	<i>I</i> _{sc,max} (μA/cm ²)	<i>K</i> _{Na} (mmol/l)	<i>n</i>
Proximal colon:			
Control rats (n=5)	59 (150)	18 (4)	1.7 (0.2)
Dexamethasone treated rats (n=7)	81 (10)	13 (2)	2.2 (0.2)
<i>p</i> **	NS	NS	NS
Distal colon:			
Control rats (n=6)	70 (12)	11 (1)	2.4 (0.3)
Dexamethasone treated rats (n=8)	211 (49)	14 (1)	2.3 (0.3)
<i>p</i> **	<0.05	NS	NS

*I*_{sc,max}=the apparent maximum short circuit current; *K*_{Na}=Na⁺ concentration at which slope of *I*_{sc} on Na⁺ concentration is maximal; *n* (Hill coefficient)=the minimum number of Na⁺ ions binding to each Na⁺-K⁺ pump site; *n*=number of tissues studied.

**=difference between control and dexamethasone treated tissues.

significantly (*p*<0.05) by dexamethasone treatment, although *K*_{Na} and *n* were unchanged.

Discussion

The results of this study indicate that rat colon exhibits appreciable segmental differences in Na⁺ and K⁺ transport in response to pharmacological doses of dexamethasone. In the distal colonic segment, dexamethasone induced or activated relatively large apical conductances for Na⁺ and K⁺ and increased the maximum activity of the basolateral Na⁺-K⁺ pump by 200%, an effect that has been shown to reflect increases in the area of the basolateral membrane and its number of Na⁺-K⁺ pump units.¹⁶ Thus dexamethasone induced a substantial amount of electrogenic Na⁺ absorption, and altered the K⁺ transport properties of the epithelium so as to favour enhanced K⁺ movement from plasma to lumen via transcellular and paracellular pathways. Although dexamethasone has by tradition been regarded as a glucocorticoid agonist, at high doses it produces >80% occupancy of mineralocorticoid receptors in addition to activating glucocorticoid receptors.⁸ Indeed, it is likely that stimulation of distal colonic Na⁺ and K⁺ transport by dexamethasone mainly reflects activation of mineralocorticoid receptors, as the electrical changes induced by high doses of dexamethasone resemble those induced by aldosterone rather than those induced by similar high doses of RU 28362, a specific glucocorticoid agonist for which mineralocorticoid receptors have negligible affinity.¹⁰

Although the effects of chronic dexamethasone administration on Na⁺ and K⁺ transport across rat distal colon have been described previously,^{9,10} the present study indicates that the proximal colon responded quite differently to a combination of mineralocorticoid and glucocorticoid receptor activation by dexamethasone. Compared with control animals, dexamethasone induced an appreciable increase in *G*_i in the proximal colon, but the associated increase in *I*_{sc} was less than that induced in the distal colon (58 μA/cm² versus 566 μA/cm², Table I). The insensitivity of dexamethasone treated proximal colon to amiloride and TEA (Fig 1) suggests that the dexamethasone induced increases in *G*_i and *I*_{sc} observed in this segment reflected stimulation of an electrogenic ion transport process(es) other than electrogenic Na⁺ absorption (or electrogenic K⁺ secretion). The inability of dexamethasone to enhance basolateral Na⁺-K⁺ pump activity in

the proximal colon is also consistent with the glucocorticoid's lack of effect on electrogenic cation transport in this segment. This notion is supported by other studies in rat proximal colon, which have shown that mineralocorticoid receptor activation by aldosterone stimulates electroneutral Na-Cl (not electrogenic Na⁺) absorption,^{17,18} and an active K⁺ secretory process which is less noticeable than that stimulated in rat distal colon.^{18,19} In contrast, the specific glucocorticoid receptor agonist RU 28362 (which also stimulates electroneutral Na-Cl absorption) has no effect on net K⁺ secretion.¹⁸ Thus, the results of the present and these other studies suggest that dexamethasone activated both mineralocorticoid and glucocorticoid receptors in rat proximal colon, resulting in the stimulation of electroneutral Na-Cl absorption and a small K⁺ secretory response which could not be detected by electrical measurements alone.

The present study also indicates that the Cl⁻ channel blocker DPC abolished the high amiloride insensitive *I*_{sc} (139 (16) μA/cm²) in dexamethasone treated proximal colon, but did not change the low amiloride-insensitive *I*_{sc} (30 (6) μA/cm²) in dexamethasone treated distal colon. This segmental difference suggests that dexamethasone induced a significant electrogenic Cl⁻ secretory process which was limited to the proximal colon. It should be noted that high doses of the glucocorticoid methylprednisolone also stimulate Cl⁻ secretion in rat distal ileum²⁰ and HCO₃⁻ secretion in rabbit distal ileum.²¹ Although Cl⁻ and HCO₃⁻ (residual) fluxes were not measured here, it is possible that the dexamethasone induced, DPC sensitive apical Cl⁻ conductance in proximal colon also had a degree of selectivity for HCO₃⁻.

The spectrum of Na⁺ transport processes present in rat colon differs from that in human colon. The predominant Na⁺ transport processes in rat colon are electroneutral Na⁺-H⁺ exchange in the proximal segment¹⁷ and electroneutral Na-Cl absorption in the distal segment.^{9,22} In contrast, there is appreciable electroneutral Na-Cl absorption in the proximal (ascending), transverse, and distal (descending) segments of human colon.^{23,24} In addition, electrogenic Na⁺ absorption is present throughout human colon,⁷ although this process only becomes noticeably amiloride sensitive distal to the splenic flexure.^{7,25} Despite these segmental and species differences in baseline Na⁺ transport mechanisms, there is evidence that suggests that similar changes in colonic Na⁺ transport may be induced in rat and human colon by glucocorticoid agonists that activate both glucocorticoid and mineralocorticoid receptors – for example dexamethasone, and methylprednisolone and hydrocortisone, both of which are used commonly in the treatment of inflammatory bowel disease. Thus, in rat proximal colon, glucocorticoid and mineralocorticoid receptor activation stimulates electroneutral Na-Cl absorption without the appearance of amiloride sensitive electrogenic Na⁺ absorption.¹⁸ It seems likely that a similar response may occur in human proximal colon, which is normally characterised by electroneutral Na-Cl absorption^{23,24} and where purely mineralocorticoid receptor activation by aldosterone fails

to induce amiloride sensitive electrogenic Na^+ absorption.⁷ In contrast, in rat distal colon and human distal colon and rectum, glucocorticoid agonists – for example dexamethasone, methylprednisolone, hydrocortisone – stimulate amiloride sensitive electrogenic Na^+ absorption via mineralocorticoid receptors^{4,10} and may also enhance electroneutral Na-Cl absorption via glucocorticoid receptors.¹⁹

In summary, it seems likely that systemically administered glucocorticoids reduce diarrhoea in active colitis by exerting direct effects on Na^+ (and hence water) absorption throughout the colon, as well as by their better recognised anti-inflammatory action. Glucocorticoids may stimulate Na^+ absorption in normal and inflamed distal colon and rectum⁴ through a combination of glucocorticoid receptor activation (enhancing electroneutral Na-Cl absorption) and crossover binding to mineralocorticoid receptors (enhancing electrogenic Na^+ absorption). In human proximal colon, glucocorticoids may only stimulate electroneutral Na-Cl absorption, despite binding to both types of corticosteroid receptor.

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