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IN VITRO EFFECTS OF DEXAMETHASONE ON SODIUM TRANSPORT ACROSS RAT COLON

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SUMMARY

1. The *in vitro* effects of dexamethasone on Na^+ transport across the colon descendens from normal rats was investigated. Amiloride was used at two concentrations, 10 μM and 1 mM, to differentially inhibit the transport of Na^+ across the colon. The colon descendens from each rat was divided into four segments and Na^+ unidirectional fluxes before and 7 h after the addition of dexamethasone (10^{-6} M) were determined under short-circuit conditions.

2. Base-line J^{Na}_{net} (net flux of Na^+) was twice as high in the proximal segment as in the distal segment. The two middle segments had intermediate rates of Na^+ transport. J^{Na}_{net} in control tissue was unaffected by 10 μM-amiloride but was completely inhibited by 1 mM-amiloride. In control tissue, amiloride at either 10 μM or 1 mM had no effect on the transmural potential difference (p.d.), the transmural conductance (G_t) or the short-circuit current (I_{sc}).

3. Dexamethasone caused a time-dependent increase in the p.d. and in the I_{sc} in all four segments of the colon. The increase in the p.d. and I_{sc} was greatest in the most distal segment and less in each of the successive more proximal segments. This segmental difference along the colon was observed in tissue from all animals studied ($n > 30$).

4. The increase in p.d. and I_{sc} caused by dexamethasone was accompanied by an increase in J^{Na}_{net} to the same maximum rate of 14 μequiv cm^{-2} h^{-1} in each segment. In the distal segment the increase in J^{Na}_{net} was fully accounted for by the appearance of an electrogenic Na^+ transport mechanism that could be inhibited by 10 μM-amiloride. In the proximal segment dexamethasone caused the appearance of electrogenic Na^+ transport, inhibited by 10 μM-amiloride, and enhanced Na^+ transport by an electroneutral mechanism that could be inhibited by 1 mM but not by 10 μM-amiloride. These results after acute exposure to dexamethasone *in vitro* are compared with the reported effects on the colonic mucosa after pre-treatment of the animals with corticoids.

INTRODUCTION

The colon descendens from normal rats absorbs Na^+ by an electroneutral, Cl^--dependent mechanism (Binder & Rawlins, 1973; Bridges, Nell & Rummel, 1983;

Perrone & Jenks, 1984). It is unclear at this time whether Na^+ transport by this mechanism is mediated by a NaCl co-transporter or parellel Na^+–H^+ and Cl^-–HCO_3^- ion exchangers. This electroneutral, Cl^--dependent mechanism, as shown in this study, is completely inhibited by 1 mM but unaffected by 10 μM-amiloride as previously reported (Bridges, Rummel & Wollenberg, 1984; Perrone & Jenks, 1984). For simplification this electroneutral, Cl^--dependent Na^+ transport mechanism will be referred to as the amiloride-'insensitive' mechanism. Prolonged treatment (> 3 days) of a rat with mineralocorticoids or high doses of glucocorticoids results in a time-dependent loss of the amiloride- 'insensitive' mechanism and the appearance of an electrogenic, Cl^--independent Na^+ transport mechanism that is completely inhibited by 10 μM-amiloride (Edmonds, 1981; Bridges *et al.* 1984; Perrone, Alexander, Bengele & Schwartz, 1984). The latter will be referred to as the amiloride-sensitive mechanism. How the corticoids cause this transition in Na^+ transport from an amiloride-'insensitive' to an amiloride-sensitive mechanism is unknown. The experiments described below were designed to investigate the *in vitro* effects of dexamethasone, a potent long-lasting glucocorticoid, on Na^+ transport across the isolated mucosa of colon descendens from normal rats. The results will show that dexamethasone can cause the appearance of the amiloride-sensitive transport mechanism *in vitro*. The distal part of colon descendens was the most responsive part of the colon to this effect of dexamethasone. In contrast to the results from rats treated for several days, the amiloride-'insensitive' mechanism was not suppressed by acute exposure to dexamethasone *in vitro*. Instead Na^+ transport by the amiloride-'insensitive' mechanism was actually enhanced by dexamethasone in the proximal part of colon descendens and was unaffected in the distal part of colon descendens.

METHODS

Animals

Laboratory-bred female Sprague–Dawley rats 200–220 g body weight were used. They were housed in wire-bottom cages and had free access to food (Altromin Diet No. 1320, Lage, F.R.G.) and water until the time of the experiments.

Tissue preparation

Four segments of colon were prepared from each rat. The colon was cut from the animal at the distal end as close to the pelvic brim as possible and approximately 8–10 cm from the anus at the proximal end. The length of colon between a large lymph node at the distal end and several smaller lymph nodes at the proximal end was used. This portion of the rat colon is approximately 6–8 cm long. The serosa, muscularis propria, submucosa and longitudinal muscle layer of muscularis mucosae were removed leaving the circular muscle layer of muscularis mucosae and the mucosa as previously described (Andres, Bock, Bridges, Rummel & Schreiner, 1985). This length of colon was then cut into four segments. These will be referred to as segments A–D with segment A corresponding to the proximal segment. Each segment was mounted as a flat sheet between two halves of a Ussing chamber. The mucosa, exposed surface area 0·5 cm^2, was sealed to the chamber with silicon grease on both the mucosal and serosal surfaces. The tissues were bathed on each side with 4 ml solution and maintained at 37 °C. The chambers were fitted with small condensers to diminish evaporative fluid loss. The exposed centres of the four segments were approximately 1·5 cm apart. The two middle segments (B and C) of this study correspond to the region of the colon used in previous reports from this laboratory.

Electrical and ion flux measurements

Agar bridges, made with 4 g agar 100 ml^{-1} bathing solution, were positioned near each surface of the tissue and at opposite ends of the chamber. Calomel electrodes and Ag–AgCl electrodes in saturated KCl were connected via the agar bridges to measure the transmural potential difference (p.d.) and to pass direct current, respectively. Electrical measurements were continuously obtained with the aid of an automatic computer-controlled voltage-clamp device (AC-microclamp, Aachen, F.R.G.). The offset potential and solution resistance were measured before mounting the tissue and automatically corrected for. Every 5 s the tissue was alternately pulsed with a (+) or (−) 25 μA pulse of 1 s duration. After a 0·5 s delay the displacement in the p.d. caused by the pulse was measured and from the change in p.d. and pulse amplitude, transmural conductance (G_t) was obtained. This procedure was used for both open- and short-circuited conditions. Thus under open-circuit conditions the open-circuit p.d. and G_t were measured and from these values a calculated short-circuit current (I_{sc}) was obtained. Under short-circuit conditions the I_{sc} and G_t were measured and from these values a calculated p.d. was obtained. Control experiments demonstrated that both normal and dexamethasone-treated tissues behaved throughout the experiment as an Ohmic resistor over a voltage range of 0 to +75 mV thus validating this protocol for obtaining the values of the electrical parameters. All three parameters, p.d., G_t and I_{sc} were recorded on a digital printer each minute. In addition either p.d. or I_{sc} were continuously recorded on a chart recorder.

Unidirectional Na^+ flux measurements were made under short-circuit conditions. 20 min after the tissue was mounted $^{22}Na^+$ was added to the bath solution on one side of the tissue. After an additional 20–30 min, by which time the isotope flux had reached a steady state, samples (two of 0·25 ml replaced by an equal volume of unlabelled solution) were taken from the unlabelled side. After an additional 30 min a second set of samples was taken. This initial flux measurement was performed on all tissues and will be referred to as the time zero flux measurement. In some experiments short circuiting was then discontinued and the tissue incubated under open-circuit conditions. Dexamethasone or solvent was then added to the mucosal and serosal solutions. After 7 h the solution on the unlabelled side was exchanged with fresh unlabelled solution. The tissue was again short-circuited and additional ion-flux measurements, each for a 30 min period, were performed. Samples were counted in a Philips liquid scintillation spectrometer. The unidirectional Na^+ fluxes were calculated using standard equations and expressed as μequiv cm^{-2} h^{-1}.

Solutions

The bathing solution contained (mM): NaCl, 107; KCl, 4·5; $NaHCO_3$, 25; Na_2HPO_4, 1·8; NaH_2PO_4, 0·2; $CaCl_2$, 1·25; $MgSO_4$, 1·0; glucose, 12. The solution was gassed with 5% CO_2 in O_2 and had a pH of 7·4. A stock solution of 10 mM-dexamethasone (Hoechst, Frankfurt, F.R.G.) in ethanol was prepared and diluted with bathing solution as necessary. Control tissues received an equivalent amount of ethanol. Amiloride was obtained from Merck, Sharp and Dohme Research Laboratories (Rahway, NJ, U.S.A.). $^{22}Na^+$ was obtained from New England Nuclear (Dreieich, F.R.G.).

Statistics

Results are given as the mean ± one standard error of the mean (S.E.). Significances of differences were tested using a two-tailed Student's *t* test. Paired or unpaired tests were used.

RESULTS

Effects of dexamethasone on the electrical parameters

The time course of the effect of dexamethasone (10^{-6} M) on the p.d. across the four different segments of rat colon descendens from a typical experiment is shown in Fig. 1. After a lag period dexamethasone caused a time-dependent increase in the p.d. across all four segments. This lag period tended to be shorter in the distal segments (approximately 90 min) and more prolonged (approximately 180 min) in the proximal segments. The p.d. increased slowly at first and then more rapidly there-

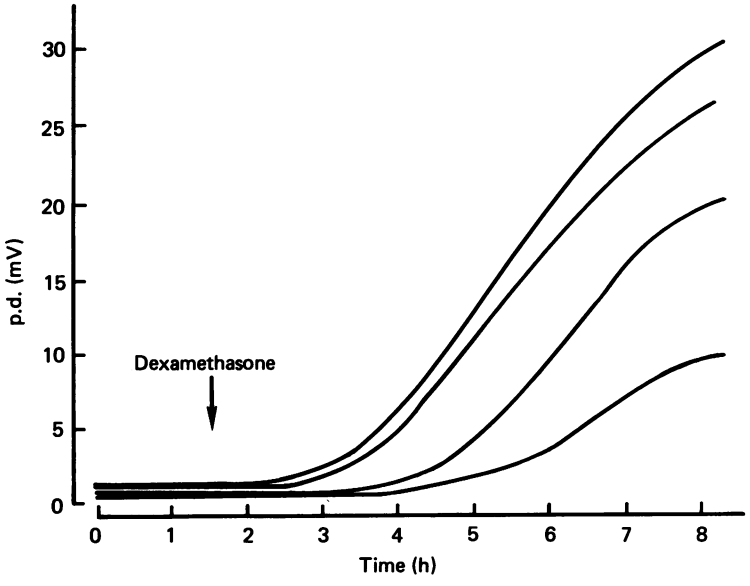

Fig. 1. Time course of the effect of dexamethasone on the p.d. across the four segments of colon descendens from one rat. Dexamethasone (10^{-6} M) was added after 90 min (arrow). The upper curve corresponds to the most distal segment and the lower curve to the most proximal segment of the colon descendens.

after. In tissue from most, but not all, animals a plateau was reached approximately 7–8 h after adding the dexamethasone. The increase in the p.d. was greatest in the most distal segment and less substantial in each of the successive more proximal segments. This segmental difference along the colon was observed in all animals studied ($n > 30$). A similar segmental heterogeneity along the colon was observed by Fromm & Hegel (1978) with the perfused colon in response to prolonged anaesthesia presumably due to elevated plasma levels of corticoids. The effects of dexamethasone on the electrical parameters across the proximal and distal segments of the colon from several experiments are summarized in Fig. 2. Base-line values of the electrical parameters in the two segments were nearly equal with the exception of a slightly higher G_t in the proximal segments. Dexamethasone caused a nearly proportional increase in the p.d. and the I_{sc} in both segments. In the proximal segment there was no significant change in the G_t but in the distal segment dexamethasone caused a significant increase in the G_t ($P > 0{\cdot}02$). The values of the electrical parameters across control tissue receiving only the solvent were unchanged over this 8 h period (data not shown).

Effect of dexamethasone on sodium transport

The net flux of Na^+ (J^{Na}_{net}) for control and dexamethasone-treated tissues is summarized in Table 1. Only the values for the most proximal and distal segments are given. The serosal to mucosal flux of Na^+ was $4{\cdot}4 \pm 0{\cdot}30$ μequiv cm^{-2} h^{-1} ($n = 8$) in the proximal segment and $2{\cdot}8 \pm 0{\cdot}25$ μequiv cm^{-2} h^{-1} ($n = 8$) in the distal segment. It was nearly constant with time and was unaffected by dexamethasone treatment

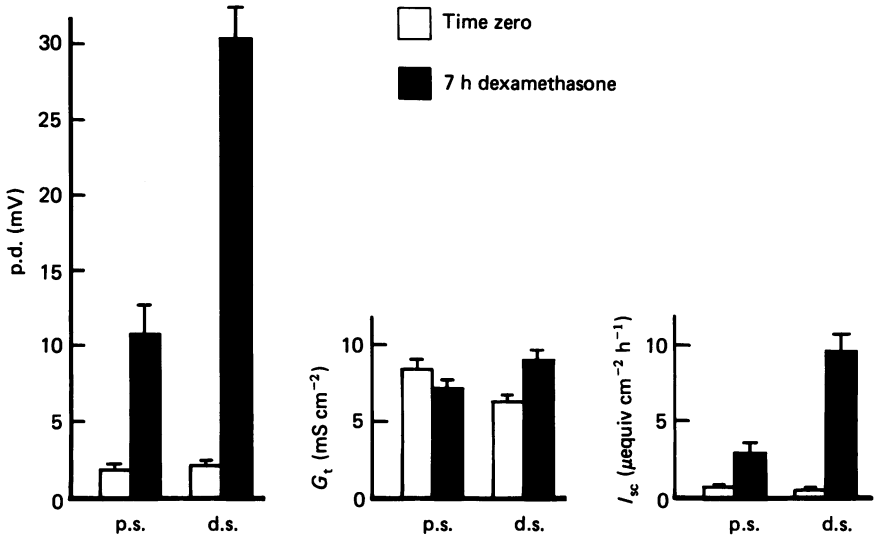

Fig. 2. Effect of dexamethasone on the electrical parameters across the proximal (p.s.) and distal (d.s.) segments of rat colon descendens. The mean ± the S.E. of the mean (n = 12 segments) is shown before (open bars) and 7 h after (filled bars) the addition of dexamethasone (10^{-6} M).

or amiloride. Therefore the changes in J_{net}^{Na} due to dexamethasone treatment or amiloride were due solely to changes in the mucosal to serosal flux of Na^+ (J_{ms}^{Na}). Base-line (time zero) J_{net}^{Na} was nearly twice as high in the proximal segment than in the distal segment ($P > 0{\cdot}02$). J_{net}^{Na} in the two middle segments was between these two values (B, $6{\cdot}9 \pm 0{\cdot}69$; C, $5{\cdot}5 \pm 0{\cdot}70$ μequiv cm^{-2} h^{-1}; $n = 6$). Remarkably, in control tissue J_{net}^{Na} did not decrease appreciably after more than 7 h incubation *in vitro*. In control tissue Na^+ transport was unaffected by 10 μM-amiloride but was nearly completely inhibited by 1 mM-amiloride. This inhibition of J_{net}^{Na} by 1 mM-amiloride was due solely to a decrease in J_{ms}^{Na}. The I_{sc} across control tissue was unaffected by either 10 μM- or 1 mM-amiloride (e.g. distal segment: control, $0{\cdot}5 \pm 0{\cdot}08$ μequiv cm^{-2} h^{-1}; 10 μM-amiloride, $0{\cdot}4 \pm 0{\cdot}06$ μequiv cm^{-2} h^{-1}; 1 mM-amiloride, $0{\cdot}6 \pm 0{\cdot}08$ μequiv cm^{-2} h^{-1}; $n = 6$). The effect of amiloride at low and high concentrations was also tested on control tissue which had not been incubated for 7 h *in vitro* and similar results were obtained (data not shown). Several additional concentrations of amiloride were also tested. The concentration of amiloride causing a half-maximal inhibition of J_{ms}^{Na} was approximately 0·4 mM and was the same for both the proximal and distal segments of the colon.

J_{net}^{Na} across tissue treated *in vitro* with dexamethasone for 7 h was significantly increased in both the proximal and distal segments. Dexamethasone caused an increase in J_{net}^{Na} of 6 μequiv cm^{-2} h^{-1} in the proximal segment and 10 μequiv cm^{-2} h^{-1} in the distal segment causing J_{net}^{Na} to be approximately the same value in the two segments. Na^+ transport in the two middle segments was increased by dexamethasone to the same value (B, $14{\cdot}0 \pm 0{\cdot}88$; C, $14{\cdot}2 \pm 0{\cdot}97$ μequiv cm^{-2} h^{-1}; $n = 6$). The increase in J_{net}^{Na} caused by dexamethasone was in all segments due to

TABLE 1. Net Na^+ transport in control and dexamethasone-treated proximal and distal segments of rat colon descendens

	Time 0 h	Time 7 h	10 μM-amiloride	1 mM-amiloride
Control				
Proximal segment	8·1±0·75	7·4±1·36	7·1±1·36	0·7±1·05
Distal segment	4·8±0·55	3·6±0·42	3·2±0·42	0·2±0·58
Dexamethasone				
Proximal segment	7·9±0·82	13·9±0·91	10·8±1·09	1·6±0·16
Distal segment	4·6±0·57	14·4±0·72	4·5±1·13	0·8±0·7

Results are means±S.E. of means; $n = 6$ in each group. Net Na^+ transport was calculated from the difference in the mean unidirectional fluxes measured on separate pieces of tissue under the same conditions. A pre-treatment (time 0 h) flux measurement was made and thereafter dexamethasone or solvent was added. 7 h later additional flux measurements were made in succession: without amiloride (time 7 h), with 10 μM-amiloride and with 1 mM-amiloride. In every case fluxes were determined under short-circuit conditions. Units of μequiv cm^{-2} h^{-1} throughout.

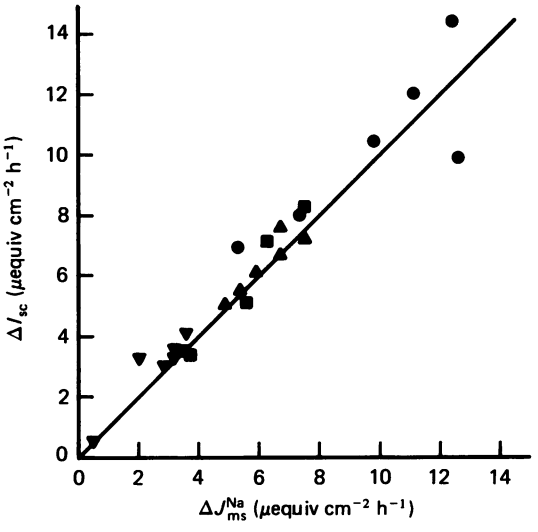

Fig. 3. The effect of 10 μM-amiloride on ΔI_{sc} and ΔJ^{Na}_{ms} across the four segments of rat colon descendens treated with dexamethasone for 7 h. The continuous line has a slope of one. The mean values for each segment were: A, $\Delta J^{Na}_{ms} = 3·2$, $\Delta I_{sc} = 3·3$; B, $\Delta J^{Na}_{ms} = 4·6$, $\Delta I_{sc} = 4·8$; C, $\Delta J^{Na}_{ms} = 6·3$, $\Delta I_{sc} = 6·5$; D, $\Delta J^{Na}_{ms} = 10·0$, $\Delta I_{sc} = 10·3$ μequiv cm^{-2} h^{-1}. Symbols represent segments A (▼), B (▲), C (■) and D (●).

an increase in J^{Na}_{ms}. In the proximal segment 10 μM-amiloride inhibited only a portion (approximately 50%) of the increase in J^{Na}_{ms} caused by dexamethasone. Thus J^{Na}_{net} in the proximal segment was still significantly higher after the addition of 10 μM-amiloride when compared with the time zero value. In contrast, in the distal segment 10 μM-amiloride completely inhibited the increase in J^{Na}_{ms} caused by dexamethasone. Thus J^{Na}_{net} in the distal segment was reduced after the addition of 10 μM-amiloride to the time zero value. In the two middle segments 10 μM-amiloride inhibited 65% in segment B and 75% in segment C of the increase in J^{Na}_{ms} caused by dexamethasone. In all segments treated with dexamethasone the decrease in

J^{Na}_{net} caused by 10 μM-amiloride was due to a decrease in J^{Na}_{ms}. The net transport of Na^+ in all segments treated with dexamethasone was completely inhibited by 1 mM-amiloride and this too was due to a decrease in J^{Na}_{ms}.

The increase in J^{Na}_{ms} caused by dexamethasone was accompanied by an increase in the I_{sc}. The increase in I_{sc} was, however, a measure of only that portion of the increase in J^{Na}_{ms} that could be inhibited by 10 μM-amiloride. As shown in Fig. 3, 10 μM-amiloride caused nearly an equivalent change in I_{sc} and J^{Na}_{ms} in all four segments. The subsequent increase in the amiloride concentration to 1 mM, although nearly completely inhibiting J^{Na}_{net}, was without any further effect on I_{sc}.

DISCUSSION

In this study amiloride was used at two concentrations to differentially inhibit the transport of Na^+ across the rat colon. At a low concentration (10 μM) it seems clear that amiloride inhibits an electrogenic Na^+ transport mechanism. This electrogenic mechanism is seen in the rat colon only after treatment with corticoids. The half-maximal effective concentration of amiloride inhibition of Na^+ transport across the colon from dexamethasone-treated rats was 0·3 μM (Bridges *et al.* 1984). In tissue treated with dexamethasone for 7 h *in vitro* the half maximal effective concentration of amiloride causing a decrease in I_{sc} was 0·37 μM ($n = 6$) and was the same for both the proximal and distal segments. These values are in good agreement with the inhibition constant (K_i) reported for the effect of amiloride on electrogenic Na^+ transport across the amphibian skin and urinary bladder (Benos, 1982; Cuthbert & Fanelli, 1978). In the colon from normal rats 10 μM-amiloride was without effect on Na^+ transport. However, as shown in this study, at a one-hundredfold higher concentration (1 mM) amiloride nearly completely inhibited the transport of Na^+ across the colon from normal rats. This inhibition was unaccompanied by any change in the p.d., G_t or I_{sc}. These results further demonstrate the electroneutral nature of the Na^+ transport mechanism in colon from normal rats.

Prolonged treatment of a rat with corticoids or manipulations that lead to an increase in the plasma levels of corticoids (e.g. low-Na^+, high-K^+ diets) have been shown to cause a time-dependent appearance of an electrogenic Na^+ transport mechanism and the disappearance of the electroneutral Na^+ transport mechanism in rat colon descendens (Edmonds, 1981; Bridges *et al.* 1984; Perrone *et al.* 1984). One intent of this study was to determine whether these effects could be demonstrated *in vitro*. The results demonstrate that dexamethasone, a long-lasting glucocorticoid, can cause a time-dependent appearance of electrogenic Na^+ transport in the normal rat colon incubated *in vitro*. Jorkasky, Cox & Feldman (1985) have also recently reported that dexamethasone, as well as aldosterone, can cause the appearance of electrogenic Na^+ transport in the distal colon descendens of the rat perfused *in vitro*. The time course of this effect was quite similar to the time course reported for the effect of corticoids on the toad urinary bladder and frog skin (Garty, 1986). The underlying cellular mechanisms leading to the appearance of electrogenic Na^+ transport in the rat colon is thus likely to be the same as in these other epithelia.

The cellular mechanism of action of the corticoids has been most extensively studied in the toad urinary bladder. In this *in vitro* model of the mammalian nephron

corticoid-stimulated Na^+ transport is dependent on genetic de-repression and new protein synthesis (for references see review of Cox & Geheb, 1984; Garty, 1986). The newly synthesized proteins are thought to do one or all of the following: (1) facilitate the entry of Na^+ into the cell at the apical membrane (the 'permease' hypothesis), (2) increase the availability of adenosine 5′-triphosphate (ATP) by stimulating mitochondrial oxidative phosphorylation, thus providing more energy for the Na^+ pump (the 'metabolic' hypothesis) and (3) facilitate the extrusion of Na^+ from the cell by activating the basolateral Na^+ pumps or somehow form new basolateral Na^+ pumps (the 'pump' hypothesis). In the rat colon, unlike in the toad urinary bladder, base-line Na^+ transport is not mediated by an electrogenic, amiloride-sensitive mechanism. Thus in the distal segment of rat colon descendens it can be concluded with greater certainty that at least one of the effects, if not the primary effect, of the corticoids is the introduction of an amiloride-sensitive Na^+ transporter into the apical membrane. It is uncertain whether this effect of dexamethasone in the rat colon is due to the activation of a pre-existing apical membrane protein or the *de novo* synthesis of a new protein.

It is also clear from the results reported here with the rat colon that in addition to causing the appearance of an electrogenic Na^+ transport mechanism dexamethasone has other effects as well. In contrast to the effects of corticoids in rats treated for several days, acute exposure to dexamethasone *in vitro* did not suppress the electroneutral amiloride-'insensitive' Na^+ transport mechanism. Instead dexamethasone actually enhanced Na^+ transport by this mechanism in the proximal segment of colon descendens and was without effect on the distal segment. These results suggest that the suppression of amiloride-'insensitive' mechanism in treated rats is due to more prolonged effects of the corticoids. The suppression may result from the prolonged exposure to the corticoids directly or to a secondary effect of prolonged treatment, perhaps mediated by another endogenous agent.

The enhancement in amiloride-'insensitive' Na^+ transport in the proximal segment of colon descendens also required several hours to develop. Sodium flux measurements in both the proximal and distal segments 90 min after the addition of dexamethasone revealed no change in Na^+ transport and after 3 h only partial increase when compared to the effect of dexamethasone after 7 h (data not shown). In each of the four segments of the rat colon dexamethasone increased Na^+ transport to approximately 14 μequiv cm^{-2} h^{-1}. The ratio of amiloride-sensitive, electrogenic Na^+ transport to amiloride-'insensitive', electroneutral Na^+ transport was highest in the most distal segment and lowest in the most proximal segment. These different effects of dexamethasone in each of the different segments would appear, however, to require the same amount of time to develop. How dexamethasone enhances both amiloride-'insensitive' and amiloride-sensitive Na^+ transport in the proximal segment while causing only an appearance of the amiloride-sensitive mechanism in the distal segment is of course unknown. What is apparent though is that the response along the colon to dexamethasone is fixed by some pre-set gradient of determinants that results in the expression of these segmental differences. The fact that despite these segmental differences the same maximum rate of Na^+ transport is achieved in each of the four segments may indicate that one step in the transport of Na^+, perhaps the exit of Na^+ from the cell, becomes rate limiting. Further studies comparing these two

different segments of rat colon descendens should prove useful in explaining how corticoids enhance Na^+ transport in the colon and in cortoid-sensitive epithelia in general.

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