Physiologic and Pharmacologic Effects of Glucocorticoids on Ion Transport across Rabbit Ileal Mucosa In Vitro

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ABSTRACT Physiologic and pharmacologic effects of glucocorticoids on ileal ion transport were examined in vitro. Tissues were obtained from the three following groups of rabbits: (*a*) normal; (*b*) glucocorticoid deficient, which were treated with aminoglutethimide (AG), 100 mg twice daily for 3 d, with a resulting marked reduction in urinary cortisol excretion but no decrease in urinary aldosterone; and (*c*) methylprednisolone-treated (MP), 40 mg daily for 2 d. Transileal NaCl fluxes were measured with radioisotopes under short-circuit conditions, and the net HCO_3 flux was assumed equal to that portion of the short-circuit current (I_{sc}) not accounted for by Na and Cl. In NaCl Ringer's solution containing 25 mM HCO_3 (pH 7.4), normals absorbed both Na and Cl and secreted HCO_3; the I_{sc} was greater in both AG and MP groups than in normals; in the AG group, no Na was absorbed, and Cl as well as HCO_3 was secreted; in the MP group, more Na was absorbed and more HCO_3 secreted than in normals. Addition of glucose to the luminal side caused similar increments in I_{sc} in all three groups, suggesting similar rates of Na-coupled glucose absorption. Secretory response was assessed with a maximal secretory simulus (8-Br-cAMP) and also a submaximal, cGMP-related secretory stimulus (*Escherichia coli* heat-stable enterotoxin). After addition of 8-Br-cAMP, the rates of net Cl secretion were similar in all three groups, suggesting no effect of glucocorticoids on maximal secretory capacity. Because the AG group was already secreting Cl, however, the cAMP-induced change in net Cl flux was least in this group. After addition of heat-stable enterotoxin, there were similar changes in net Cl flux in all three groups. To examine specifically Cl-independent, electrogenic Na transport, we used a 10 mM HCO_3, Cl-free SO_4-Ringer (ph 7.2) in which net Na absorption was previously shown to be equal to the I_{sc}. Under these conditions, I_{sc} was greatest in the MP group and least in the AG group. In vitro addition of hydrocortisone, 50 μg/ml, to AG tissues had no effect on Cl fluxes or I_{sc} over a 3.5-h period. No differences among groups were observed with respect to morphology, electrical resistance, or cGMP concentration. We conclude that (*a*) the effect of glucocorticoid deficiency is similar to that of a submaximal secretory stimulus in that Na absorption is inhibited and some Cl secretion develops; (*b*) electrogenic Na absorption is depressed in glucocorticoid deficiency and enhanced in glucocorticoid excess; (*c*) glucocorticoid excess increases HCO_3 secretion; and (*d*) glucocorticoid status does not affect maximal secretory capacity.

INTRODUCTION

Glucocorticoids have been used to treat diarrhea associated with inflammatory bowel disease, nontropical sprue, and hormone-producing tumors (1). Although in some of these circumstances the antidiarrheal effects of the glucocorticoids may be caused by suppression of the underlying disease process, there is also evidence from animal studies to suggest that glucocorticoids have a direct effect on intestinal salt and water absorption: pharmacologic doses of methylprednisolone have been shown to increase Na, Cl, and water absorption in vivo in both small intestine and colon of the rat (2). In contrast, mineralocorticoids appear to stimulate salt and water absorption only in the colon (2–4). We are not aware of any studies which evaluate effects of physiologic concentrations of glucocorticoids on small intestinal salt and water transport. Because watery

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diarrhea is a recognized complication of Addison's disease (5), there is a reason to suspect such effects.

This study was undertaken to evaluate effects of both physiological and pharmacologic concentrations of glucocorticoids on active ion transport in rabbit ileum. Isolated glucocorticoid deficiency was induced in rabbits by 3 d of treatment with aminoglutethemide (AG).[1] Glucocorticoid excess was produced by 2 d of treatment with methylprednisolone (MP). Ion transport properties were then examined in vitro.

METHODS

Treatment groups. New Zealand white male rabbits (2–3 kg) were fed standard rabbit chow and water ad lib. Rabbits from each shipment were randomly assigned to one of three groups: untreated controls; steroid-treated (MP), which received 40 mg MP succinate i.m. daily for 2 d; and steroid-depleted (AG), which received AG phosphate 100 mg i.m. twice daily for 3 d. Rabbits from each group were used in rotation to minimize temporal or other incidental differences among groups.

15–20 h after the last injection of AG or MP, rabbits were killed by cervical dislocation, and the distal ileum was removed rapidly, opened along its mesenteric border, and rinsed clean of luminal contents with cold Ringer's solution containing, in mmol/liter, NaCl, 114; KCl, 5; Na_2HPO_4, 1.65; NaH_2PO_4, 0.3; $CaCl_2$, 1.25; $MgCl_2$, 1.1; and $NaHCO_3$, 25 (standard Ringer). Before use, tissues were maintained in ice-cold Ringer with 95% O_2/5% CO_2.

The serosa and two major muscle layers were removed by placing a 10-cm sheet of ileum, serosa up, on a Plexiglass plate moistened with Ringer, making a transverse incision through the muscle layers with a razor blade, and peeling the layers off longitudinally with fine curved forceps.

Electrical and ion flux measurements. Transepithelial electrical potential difference (PD), total conductance (G_t), and short-circuit current (I_{sc}) were measured as described previously (6). Four pieces of intestinal mucosa were mounted in Ussing chambers (1.12 cm^2 exposed surface area), and bathed with 10 ml of standard Ringer on each side. Solutions were circulated by gas lift and maintained at 37°C in water jacketed reservoirs. Glucose, 10 mmol/ml, was added to the serosal medium, and an equimolar amount of mannitol was added to the mucosal medium. In some experiments, I_{sc} measurements were made in a Cl-free, low HCO_3-Ringer solution which contained, in mmol/liter: Na_2SO_4, 57; K_2SO_4, 2.5; $CaSO_4$, 1.25; $MgSO_4$, 1.1; $NaHCO_3$, 10; Na_2HPO_4, 1.65; NaH_2PO_4, 0.3; and mannitol, 62 (sulfate Ringer). The pH of this solution was 7.2.

Ion fluxes were measured over two successive periods. After the tissues had been mounted for 45–55 min, ^{22}Na and ^{36}Cl were added together to either the mucosal (m) or serosal (s) reservoirs, and the tissues were short-circuited. Tissues were paired by matching resistances. If at any time during flux measurements the resistances of paired tissues differed by more than 25%, the experiment was rejected.

Unidirectional m-to-s and s-to-m fluxes (J_{ms}, J_{sm}), and net fluxes ($J_{net} = J_{ms} - J_{sm}$) of Na and Cl were calculated from initial samples and samples taken 20 min later (period I). From these measurements, the residual ion flux ($J^R = I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$), which represents that part of the I_{sc} not attributable to the movement of Na or Cl, was calculated. In most instances duplicate paired determinations were made for each animal. These were then averaged to provide one set of results for each animal.

In some experiments a secretory stimulus, either 8-Br-cAMP (5 μmol/ml) or *Escherichia coli* heat-stable enterotoxin (ECST), 12 mouse U/ml, was added immediately after period I. 20 min later, a second 40-min flux period was begun (period II). After this, D-glucose, 10 μmol/ml, was added to the luminal side and the resulting change in I_{sc} recorded. Amiloride (0.1 μmol/ml) was added to the mucosal side after period I flux in some experiments.

Cyclic GMP assay. Six pieces of mucosa (20–60 mg each) were placed in flasks with 25 ml of standard Ringer, which were individually gassed with 95% O_2. Flasks were incubated at 37°C with shaking at 90 rpm.

After 40 min, two to four tissue samples were transferred quickly to each of two test tubes containing ice-cold 5% trichloracetic acid and 4 nCi of [^{3}H]cGMP (0.1 pmol) added as a recovery marker.

After homogenizing with a motor-driven pestle and centrifuging for 15 min at 2,000 *g*, the trichloracetic acid supernates were extracted four times with water-saturated diethyl ether and then evaporated to dryness at 60°C under a stream of nitrogen. The residues were redissolved in 0.5–1.0 ml of 100 mM sodium acetate buffer (pH 6.4), and the cGMP present in 20- to 100-μl portions was determined in duplicate by radioimmunoassay as described by Harper and Brooker (7), except that albumin was omitted from the reaction mixture. Lyophilized cGMP antibodies were dissolved in 0.5 ml H_2O and diluted with acetate buffer to bind 35–60% of the free ^{125}I-labeled ligand (anti-cGMP, 1:16,000 dilution). The trichloracetic acid precipitates were dissolved in 1 N NaOH and assayed for protein by the method of Lowry et al. (8). Results are expressed as picomoles cGMP per milligram protein.

Steroid assays. Rabbits were housed in metabolic cages for 24-h urine collections. Urine was assayed for free cortisol by a competitive protein binding assay (9) and aldosterone by radioimmunoassay (10) as previously described.

Histology. Ileal mucosa from each group of rabbits was placed in buffered 4% formalin for at least 1 wk, embedded in paraffin, cut into thin sections, and stained with hematoxylin and eosin by standard techniques. Coded slides were examined by Robert H. Riddell, a member of the faculty in Pathology at the University of Chicago. He found no differences in villus height, crypt depth, inflammatory infiltrate, or any other parameter which might differentiate the three groups.

Statistics. Student's unpaired *t* test was used for intergroup comparisons of periods I and II fluxes and responses to glucose.

Materials. The following materials were used: ^{22}Na and ^{36}Cl, New England Nuclear (Boston, Mass.); [^{25}I]cGMP, Becton-Dickinson & Co. (Rutherford, N. J.); [^{3}H]cGMP (21 Ci/mmol), Amersham Corp. (Arlington Heights, Ill.); 8-Br-cAMP, Sigma Chemical Co. (St. Louis, Mo.); MP sodium succinate, Upjohn Co. (Kalamazoo, Mich.); hydrocortisone sodium succinate, Abbott Laboratories (North Chicago, Ill.). Antibodies to cGMP were a gift of A. L. Steiner (University of Texas at Houston). Partially purified ECST prepared and assayed as previously described (11) was a gift of Walter Laird (National Bureau of Standards, Washington, D. C.). AG phosphate was obtained from Ciba-Geigy Corp., Pharmaceuticals Div. (Summit, N. J.). Amiloride was obtained from Merck, Sharp & Dohme Research Laboratories (West Point, Pa.).

[1] *Abbreviations used in this paper*: AG, aminoglutethimide; ECST, *Escherichia coli* heat-stable enterotoxin; G_t, total conductance; I_{sc} short-circuit current; J^R, residual ion flux, MP, methylprednisolone, PD, electrical potential difference.

TABLE I
Effects of Treatment with AG on Urinary Excretion of Corticosteroids

	Corticosteroid output on day:				
	0	1	2	3	4
			μg/24 h		
Cortisol	1.15±0.20 (6)	0 (3)	0 (6)	0 (5)	1.0±0.50 (2)
Aldosterone	0.18±0.06 (5)	0.59±0.22 (4)	0.27±0.08 (4)	0.18±0.04 (6)	

Values are means±SE for (*n*) rabbits. Day 0 refers to the 24 h before the first injection of AG. A value of zero implies less than 0.2 μg of cortisol.

RESULTS

Effects of AG on rabbit steroid production. AG blocks formation of pregnenolone in the steroid synthetic pathway by inhibiting 20.22 desmolase (12, 13). As shown in Table I, determinations of 24-h urinary cortisols revealed effective inhibition of glucocorticoid production for 3 d; in contrast, urinary aldosterone excretion was not reduced, even transiently. Therefore, by the time tissues were taken for in vitro studies (after 3 d), AG had effectively inhibited glucocorticoid output without blocking mineralocorticoid output.

Effect of AG added in vitro on ion transport. To determine if AG has a direct effect on ileal ion transport, sections of ileal mucosa from normal rabbits were mounted in vitro, and AG was added to the serosal side of some to yield a concentration of 0.43 mM. (This is the approximate concentration of AG after a single 100-mg injection, assuming distribution in extracellular water; because animals were killed 12 h after the last dose of AG, the actual tissue concentration at that time was undoubtedly lower.) Cl fluxes and I_{sc} were determined for the period 15–45 min after AG addition. Mean values for J^{Cl}_{net} and I_{sc} in $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ were 3.47±1.19 (1 SE) and 1.28±10.31, respectively (three rabbits). In paired control tissues, mean values for J^{Cl}_{net} and I_{sc} were 3.40±1.01 and 1.63±0.29. Thus AG does not appear to affect ileal ion transport by a direct action on the ileal mucosa.

Effect of steroid status on ion transport. Ion fluxes across ileal mucosa from the three treatment groups are shown in Table II. In the control group (normal), Na and Cl were absorbed, and HCO_3 (as measured by J^R[14]) was secreted. In the MP group, I_{sc}, J^{Na}_{net}, and J^R were significantly greater than control values. Although mean J^{Cl}_{net} was also greater, this difference was not statistically significant ($0.05 < P < 0.10$).

AG treatment abolished the normal net absorptive fluxes of Na and Cl, the latter flux becoming secretory. I_{sc} was significantly elevated, but J_R was unchanged.

There was no difference in conductance among the three experimental groups. Addition of amiloride, 0.1 μmol/ml, to the luminal side did not reduce I_{sc} in either normal or MP groups (data not shown).

With respect to unidirectional fluxes, J^{Na}_{sm} was significantly greater in the AG than in the MP group; J^{Cl}_{sm} was significantly greater in the AG than in the normal group; and J^{Cl}_{ms} was significantly smaller in the normal and AG groups than in the MP group.

In summary, (*a*) glucocorticoid deficiency (AG group) inhibited Na absorption and reversed Cl transport from absorption to a low rate of secretion. The increased I_{sc} in the AG group can be attributed to a relatively greater change in J^{Cl}_{net} than in J^{Na}_{net}. (*b*) Glucocorticoid excess (MP group), on the other hand, increased Na absorption, I_{sc} and J^R, the last change suggesting an increase in HCO_3 secretion (14). The overall net transports of ionic solutes under short-circuit conditions (J^{Na}_{net} plus J^{Cl}_{net}

TABLE II
Ion Fluxes across Ileal Mucosa from Normal Rabbits Compared with Those Treated with either MP or AG

Group	Na_{m-s}	Na_{s-m}	Na_{net}	Cl_{m-s}	Cl_{s-m}	Cl_{net}	I_{sc}	J^R	G_t
Normal, *n* = 17	15.6±0.70	13.8±0.67	1.77±0.46	11.2±0.60	9.0±0.66	2.26±0.50	1.47±0.27	1.90±0.49	28.6±0.86
MP, *n* = 19	16.5±0.82	12.3±0.69	4.14±0.66*	13.1±0.69‡	9.7±0.51	3.39±0.63	4.98±0.44*	4.21±0.62‡	28.1±0.77
AG, *n* = 18	15.6±1.03	16.0±1.21§	−0.43±0.52*‖	9.9±0.50‖	11.3±0.80‡	−1.38±0.65*‖	2.86±0.28*‖	1.92±0.78§	30.2±1.13

Values are means±1 SEM for (*n*) animals. Fluxes and I_{sc} are expressed in $\mu eq \cdot h^{-1} \cdot cm^{-2}$. G_t is expressed in $mmhos \cdot cm^{-2}$. Statistical comparisons: ‡ $P < 0.05$ and * $P < 0.01$ vs. normal group; § $P < 0.05$ and ‖ $P < 0.01$ vs. MP group.

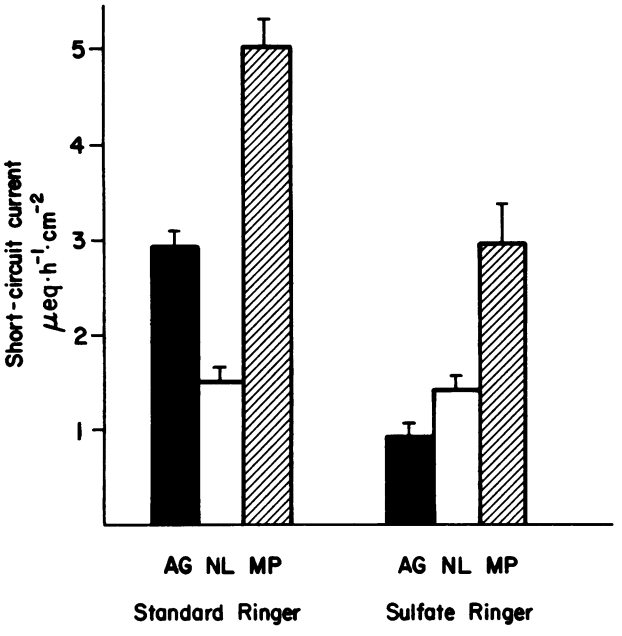

FIGURE 1 Steady-state I_{sc} of ileal mucosa from AG-treated, normal, and MP-treated rabbits. Results are shown for tissues bathed in Cl-containing and Cl-free (SO_4) Ringer solutions.

minus J^R) were −3.7, 1.7, and 3.6 in AG, normal, and MP groups, respectively. This flux was significantly altered by AG treatment ($P < 0.01$) but not by MP ($0.05 < P < 0.1$).

Effect of steroid status on I_{sc} in Cl-free, low HCO_3 Ringer. In order to evaluate further the effect of MP and AG treatments on electrogenic Na transport (see Discussion), experiments were performed in the absence of Cl and with HCO_3 concentration reduced to 10 mM (pH 7.2). Cl and HCO_3 were replaced by SO_4 and mannitol. It was previously shown (15, 16) that, under these conditions, the I_{sc} is equal to J^{Na}_{net}. Fig. 1 compares values for I_{sc} obtained in this Ringer solution to those obtained in the standard Ringer. In standard Ringer, the I_{sc} relations were MP > AG > normal. In Cl-free, low HCO_3-Ringer, this order changed to MP > normal > AG. The results suggest that glucocorticoids enhance electrogenic Na transport. As was the case in standard Ringer, addition of amiloride, 0.1 μmol/ml, to the SO_4-Ringer did not alter I_{sc} (data not shown).

Response to secretagogues. Table III lists flux changes between two sequential periods occurring either spontaneously (A, control) or after addition of secretagogues between periods (B and C).

An antiabsorptive trend from periods I to II was observed even when no secretagogue was added after period I. These spontaneous changes were approximately the same for each treatment group, and therefore differences among groups in period I were still present in period II when no secretagogue was added. It follows that the changes observed after additions of secretagogues were caused by the actions of the secretagogues, and not to spontaneous changes with time.

As shown in Table IIIB, 8-Br-cAMP induced a secretory response in each group characterized by an increase in I_{sc} and decrease in both J^{Na}_{net} and J^{Cl}_{net}. The changes in the latter fluxes were significantly smaller in the AG group. With regard to unidirectional fluxes, the change in J^{Cl}_{ms} and J^{Ns}_{ms} caused by 8-Br-cAMP were similar for all three treatment groups, but the change in J^{Na}_{sm} were smaller in the AG group. An 8-Br-cAMP induced decrease in G_t developed in both normal and AG groups, but was nearly absent in the MP group.

ECST has been previously shown to inhibit Na and Cl absorption in rabbit ileum, probably as a result of an

TABLE III
Ion Flux Changes Induced by 8-Br-cAMP and ECST

Treatment group	ΔJ_{m-s}^{Na}	ΔJ_{s-m}^{Na}	ΔJ_{net}^{Na}	ΔJ_{m-s}^{Cl}	ΔJ_{s-m}^{Cl}	ΔJ_{net}^{Cl}	ΔI_{sc}	ΔJ^R	ΔG_t
(A) Control									
Normal (4)	−0.81±0.23	0.97±1.30	−1.74±1.32	0.28±0.75	1.18±1.00	−0.90±1.33	0.91±0.44	1.58±0.58	−1.33±0.38
MP (7)	−0.32±0.60	1.05±0.29	−1.37±0.58	−0.20±0.51	1.47±0.44	−1.65±0.60	0.66±0.37	0.43±0.47	−1.28±0.70
AG (6)	−0.39±0.38	+0.83±0.64	−1.20±0.37	−0.45±0.75	1.17±0.25	−1.63±0.85	0.53±0.45	0.22±0.90	−0.80±1.14
(B) 8-Br-cAMP									
Normal (5)	−5.96±1.18	0.08±1.90	−6.04±0.86	−4.54±1.26	4.15±1.58	−8.69±1.10	4.49±0.32	1.97±1.04	−7.43±0.86
MP (5)	−3.29±0.31	1.13±0.72	−4.42±0.94	−4.19±0.63	2.86±0.85	−7.08±1.24	4.05±1.02	1.53±1.11	−1.72±0.52*
AG (6)	−4.86±0.60	−2.44±0.44‡	−2.45±0.90§	−3.00±0.32	0.34±1.06	−3.35±1.09§‖	3.11±0.62	2.07±1.44	−6.56±0.69‡
(C) ECST									
Normal (7)	−1.69±0.51	0.80±0.45	−2.51±0.86	−1.42±0.59	1.97±0.38	−3.39±0.68	1.63±0.43	1.00±0.90	−2.17±0.52
MP (6)	−3.39±1.20	1.50±0.57	−4.90±1.27	−2.09±1.11	4.44±0.90	−6.53±1.55	2.89±0.52	1.14±1.28	−3.50±1.08
AG (5)	−2.50±1.15	1.49±1.74	−3.99±1.66	−0.08±0.52	2.17±0.47	−2.27±1.46	1.47±0.49	3.19±1.24	−3.77±0.59

ΔJ represents the difference between sequential flux measurements (Methods). Between flux periods either no secretagogue was added (A, control), or 8-Br-cAMP (B) or ECST (C) was added to the serosal reservoir. Values are means±1 SEM for (*n*) animals. Fluxes and I_{sc} are expressed in $\mu eq \cdot h^{-1} \cdot cm^{-2}$. G_t is expressed in $mmhos \cdot cm^{-2}$.

* $P < 0.01$ compared with normal.

‡ $P < 0.01$ MP vs. AG.

§ $P < 0.05$ compared with normal.

‖ $P < 0.05$ MP vs. AG.

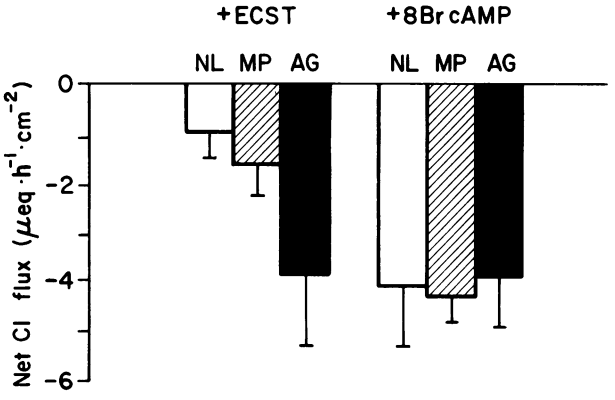

FIGURE 2 Rates of net Cl secretion after submaximal (ECST) and maximal (8-Br-cAMP) secretory stimuli added in vitro to ileal mucosa from normal, MP-treated, and AG-treated rabbits.

increase in cGMP concentration (11, 17, 18). The effects of ECST are shown in Table IIIC. Although ECST-induced changes in J^{Na}_{net}, J^{Cl}_{net} and I_{sc} appear to be greater in the MP group, there is no significant difference in the change produced by ECST among the three treatment groups.

Fig. 2 shows the actual values for J^{Cl}_{net} during period II after addition of 8-Br-cAMP or ECST. The rate of net Cl secretion produced by 8-Br-cAMP is not significantly affected by either glucocorticoid deprivation or excess. After addition of ECST, which has previously been shown to have a smaller effect on Cl secretion than does theophylline (11), significant changes in net Cl flux were observed in normal and MP groups but not in the AG group, which was already secreting Cl under basal conditions.

Effect of glucocorticoid status on mucosal cGMP concentration. Recent preliminary communications report that MP treatment of rats increases ileal mucosal cGMP concentration (19) and stimulates Cl secretion in vitro (20). Because cGMP has been postulated to mediate the action of ECST (11, 17, 18), it was suggested that the above two effects of MP treatment are causally related. Because of these observations, we determined cGMP concentrations in rabbit ileal mucosa for each treatment group. Results (±1 SEM) in pmol cGMP/mg protein were as follows: normals, 0.139±0.023 (n = 4); MP treated, 0.146±0.026 (n = 4); and AG treated, 0.180±0.024 (n = 8). Differences among groups are not statistically significant.

Effect of glucocorticoid status on the response to luminal addition of glucose. Na-coupled glucose absorption in each treatment group was estimated by measuring the I_{sc} response to the addition of glucose to the luminal reservoir. A number of studies have demonstrated the validity of using this electrical response in rabbit ileum to estimate active transport rates for sugars and neutral amino acids (21, 22).[2] Glucose (10 μmol/ml) was added immediately after completion of period II flux measurements. Responses in the presence and absence of secretagogues were similar and, therefore, have been pooled. The following I_{sc} increments ($\mu Eq \cdot h^{-1} \cdot cm^{-2}$) were recorded: normals, 1.62±0.29, n = 17; MP treated, 2.17±0.19, n = 13; and AG treated, 1.68±0.20, n = 14. Although the mean value for the MP-treated group was greater than for the other two groups, this difference is not significant. Charney et al. (2) previously showed a 75% increase in glucose absorption in the ileum of MP-treated rats. We could not confirm their in vivo result for rat ileum in our in vitro experiments with rabbit ileum, but a trend in that direction is present.

In vitro replacement of glucocorticoid. To assess whether in vitro replacement of glucocorticoid would affect ileal ion transport in tissues from glucocorticoid-deficient (AG-treated) rabbits, hydrocortisone sodium succinate, 50 μq/ml, was added to the serosal side of some tissues after basal Cl fluxes had been measured. Fluxes were again measured 3–3.5 h later, and changes in J^{Cl}_{net} in hydrocortisone-treated and control tissues were compared. Nearly identical increases in net Cl secretion developed between the two flux periods in both hydrocortisone-treated and control tissues (ΔJ^{Cl}_{net} = −1.98, 0.73, hydrocortisone-treated; and ΔJ^{Cl}_{net} = −1.72, 0.26, controls; n = 3). Significant changes in I_{sc} did not develop. Therefore, hydrocortisone does not alter ion transport within 3.5 h after its addition in vitro to ileal mucosa from AG-treated rabbits.

DISCUSSION

This study demonstrates both physiologic and pharmacologic effects of glucocorticoids in the regulation of ileal ion transport. Ileal mucosa from glucocorticoid-deficient rabbits exhibited, under short-circuit conditions, net Cl secretion and no net Na absorption. This contrasts with the in vitro behavior of ileal mucosa from control rabbits, which absorbed both Na and Cl. Glucocorticoid deficiency did not, however, impair the normal electrical response to the luminal addition of glucose. Thus normal glucocorticoid production appears necessary for salt and water absorption in the small intestine when nutrients which specifically stimulate Na absorption are not present in the lumen. This may partly explain the watery diarrhea in some patients with Addison's disease (5).

Because AG treatment markedly decreased urinary cortisol excretion without decreasing urinary aldosterone, its use provided a unique opportunity to study the effect of isolated glucocorticoid deficiency.[3] The

[2] It seems very unlikely that this relationship is altered by glucocorticoid deficiency or excess, but we cannot be certain.

[3] The possibility of extraadrenal affects of AG have been considered. This drug has been used widely specifically to in-

rise in urinary cortisol on days 3 and 4 of treatment was not unexpected, because, with continued adiministration of AG there is accelerated hepatic metabolism of AG (34) and increasing pituitary release of ACTH (14). The lack of aldosterone suppression was unexpected because similar doses administered to rats and man effectively inhibited aldosterone output (13, 35–37).[4]

Administration of pharmacologic doses of MP in vivo increased net Na absorption, PD, I_{sc}, and J^R, when these parameters were subsequently determined in vitro. Although mean J^{Cl}_{net} and the overall flux of ionic solute (J^{Na}_{net} and $J^{Cl}_{net} - J^R$) also increased, these changes were not statistically significant. With respect to Na transport and I_{sc}, these findings agree with previous in vivo observations on rat ileum (2). A comparable in vitro study with rat ileum (20) differs with both in vivo rat data and our in vitro rabbit data; although MP treatment was found to increase net Na absorption, it was also found to stimulate net Cl secretion; MP treatment was also reported to increase mucosal cGMP concentration, suggesting that this change may be responsible for the observed Cl secretion (19). We were unable to demonstrate any difference in mucosal concentrations of this cyclic nucleotide in rabbits treated with either MP or AG, and this may account for the differences between our study and these prior in vitro observations in the rat. We also found that ECST has the same secretory (antiabsorptive) effect on tissues from both normal and MP-treated rabbits. Therefore, treatment of rabbits with MP does not alter either the ileal mucosal concentration of cGMP or the response of the mucosa to a cGMP-related secretagogue.

The data presented suggest the three following principal effects of glucocorticoids on ileal ion transport: (*a*) at physiologic dosage and, to a greater extent, at pharmacologic dosage, stimulation of electrogenic Na absorption; (*b*) at physiologic dosage, inhibition of Cl secretion; and (*c*) at pharmacologic dosage, stimulation of HCO_3 secretion.

Glucocorticoid regulation of electrogenic Na absorption. Active Na absorption in rabbit ileum results from electrogenic (i.e., manifested by increases in PD and I_{sc}) and nonelectrogenic (i.e., Cl-coupled) processes (21). Glucocorticoid effects on electrogenic Na transport could not be ascertained from measurements in the standard Ringer solution because of possible contributions of Cl and HCO_3 to the I_{sc} and net Na flux. Indeed, in the standard Ringer, the I_{sc} was higher in tissues from both AG- and MP-treated rabbits than in controls. In Cl-free, low HCO_3 Ringer, however, Cl and HCO_3 net transports are eliminated, and the I_{sc} is a reliable measure of net Na absorption (15, 16). In this solution, there was a clear separation among the three treatment groups—the I_{sc} being lowest in the AG group and highest in the MP group. Thus, these differences in I_{sc} suggest that electrogenic Na absorption in the ileum is under glucocorticoid control, being diminished in glucocorticoid deficiency and enhanced in glucocorticoid excess. It should be added, however, that the effect of glucocorticoid deficiency was small relative to that of glucocorticoid excess.

A priori, active Na absorption can be increased by either increasing the Na permeability of the brush border membrane or by increasing Na pump activity (Na, K-activated ATPase) at the contraluminal membrane, or both. Aldosterone's enhancement of active Na absorption in the distal colon of the rabbit develops, at least initially, through an increase in luminal Na permeability (3); possibly a more delayed additional increase in the number of Na pumps per cell also occurs (2). Glucocorticoids at pharmacologic dosage have been shown to increase Na, K-activated ATPase activity in homogenates from both small and large intestine of the rat, but, in the colon at least, this effect does not become apparent until long after there is a demonstrable increase in transmural PD and Na absorption in vivo (40). Furthermore, Na pump capacity is not ordinarily rate-limiting in either small or large intestine because agents that act initially or exclusively at the luminal

hibit steroid synthesis, just as metyrapone has. In reviewing an extensive bibliography on AG, we noted a few effects other than steroid inhibition: (*a*) AG has anticonvulsant and sedative properties. Although the effects of neuroleptic drugs on intestinal fluid absorption have not been fully explored, those agents which have been studied have proven to be absorptive or antisecretory stimuli (see, for example, recent studies on tricyclic antidepressants [23] and enkaphalins [24, 25]. There are also scattered reports that diphenylhydantoin is an absorptive stimulus (26, 27). (*b*) There have been occasional reports that AG alters the intermediate metabolism of thyroid hormone and, less frequently, it has been found to diminish circulating levels of protein-bound iodine or T_4. This has been ascribed to a propylthiouracil effect on thyroid metabolism (28, 29). This type of metabolic block does not prevent release of preformed thyroid hormone from plentiful colloid stores in the gland, and, therefore, no significant reduction of circulating levels would be expected in acute studies such as these. (*c*) AG as a stimulatory effect on phenylethanolamine-*N*-methyl transferase activity and increases the adrenal concentration of epinephrine (30). Epinephrine, however, stimulates salt and water absorption in the intestine (14, 31), and, therefore would be expected to cause an effect opposite of that observed. (*d*) AG, like the closely related sedative glutethimide, can cause hyperplasia of hepatic smooth endoplasmic reticulum after chronic administration (32), causing accelerated metabolism of glucocorticoids (13), androgens (33), and aminoglutethimide itself (34). There is nothing to suggest that these hepatic changes would lead to intestinal secretion. Also, as shown in the present study, in vitro addition of AG does not significantly affect ileal ion transport.

[4] It is unclear whether this difference is caused by variation in treatment protocol or species. Previous studies have demonstrated that steroid metabolites beyond pregnenolone are not uniformly inhibited (35, 36, 38, 39); AG may have other inhibitory effects in steroid synthesis in addition to its action on 20.22 desmolase (27).

border, such as glucose and amphotericin, cause a rapid increase in Na transport (3, 21). Thus, while direct evidence is not available, it is likely that glucocorticoids stimulate electrogenic Na absorption in the ileum primarily by increasing the Na permeability of the brush border membrane. Both under basal conditions and with glucocorticoid enhancement, ileal Na permeability was amiloride insensitive, which contrasts with the amiloride-sensitive nature of both basal and mineralocorticoid-stimulated Na transport in distal colon (3).

It should be emphasized that, under other circumstances, increases in active Na absorption in the ileum are associated with decreases in I_{sc}. This inverse association can be produced by addition of α-adrenergic agonists (31), somatostatin (41), or enkephalins (24, 25). In all of these instances, electrically neutral NaCl absorption is enhanced and HCO_3 secretion inhibited. Similar observations have been made in rat colon in vitro (42).

Glucocorticoid regulation of Na-dependent Cl absorption and secretion. A variety of studies attest to the Na dependence of Cl transport in rabbit ileum and to its regulation by cAMP (15, 16, 21, 43). There appear to be two separable effects of cAMP on ileal ion transport: (*a*) inhibition of NaCl cotransport across the brush border with a resulting inhibition of NaCl absorption, and (*b*) stimulation of electrogenic (but Na-dependent) Cl secretion. Both effects contribute to the overall shift in electrolyte transport from absorption to secretion. Agents that increase intestinal mucosal cAMP concentration (cholera toxin (44), vasoactive intestinal peptide (45), prostaglandins (23, 41, 44), theophylline and cAMP analogues (41, 43), if added in sufficient quantity, appear to evoke a maximal secretory response. Combined addition of theophylline (10 mM) and dibutyryl cAMP (0.5 mM), for example, does not evoke a greater response than either alone (43). Furthermore, addition of the Ca ionophore, A23187 (which does not alter cAMP concentration but evokes Ca-dependent secretion) does not produce a greater effect than does addition of theophylline, nor does their combined addition evoke a larger response than theophylline alone (46). Similarly, the cGMP-related agonist ECST is less effective than theophylline or cAMP, and the effect of combined addition of ECST and theophylline does not exceed the effect of theophylline alone (11). As shown in Fig. 2, after addition of 8-Br-cAMP sufficient to evoke a maximal secretory response, net Cl fluxes were approximately the same in the three threatment groups, demonstrating that glucocorticoids do not alter maximal secretory capacity. Because this is the case, there must be some relation between the initial rate of Cl transport and the magnitude of change produced by a maximal secretory stimulus. In the AG group, basal Cl transport was already secretory, and, therefore, the change in Cl transport produced by 8-Br-cAMP was smaller than in the other two groups. ECST, on the other hand, is not a maximal secretory stimulus, and therefore the relative magnitudes of its effect are less predictable. Although it appears to have had its greatest effect on Cl transport in the MP group, which had the highest basal rate of Cl absorption, the differences among its effects in the three treatment groups are not statistically significant.

Glucocorticoid regulation of ileal HCO_3 secretion. Ileal HCO_3 secretion across short-circuited rabbit ileal mucosa has previously been shown to approximately equal the residual ion flux under a variety of conditions (14). Thus the increase in J^R noted in the MP group is almost certainly a reflection of an increase in HCO_3 secretion. AG treatment did not alter J^R, implying that physiologic amounts of glucocorticoid do not affect HCO_3 secretion. Whether or not MP also stimulates active HCO_3 secretion in vivo was not determined in the prior studies with rat ileum (2).

Because the increase in J^R noted in vitro was nearly the same as the increase in net Na absorption (compared with normals), total solute flux did not increase significantly. Assuming that water flux (not measured in vitro) is proportional to net solute flux, significant stimulation of water absorption by MP probably did not develop in vitro. In contrast, water absorption by rat ileum in vivo was found to increase with MP treatment (2). It is unclear whether this difference was caused by the contribution of the open circuit potential to net solute (and presumably net water) flux or caused rather by species variations in the relative effects of MP on active Na and HCO_3 transport rates.

How do glucocorticoids regulate intestinal ion transport? Addition of cortisol in vitro to ileum from AG-treated animals did not alter I_{sc} or Cl fluxes over a 3.5-h period. The failure of glucocorticoids to evoke short-term changes in the ileum in vitro is in contrast with the action of aldosterone, which can alter Na transport in rabbit distal colon as early as 30 min after in vitro addition (3). Dexamethesone caused a rise in rat colon PD in vivo in 3 h (40). Rat ileum, treated in vivo with MP, showed increased Na absorption after 16 h (47) (earlier time points were not obtained). The absence of a demonstrable in vitro effect in the present study may have been a result of the relative short period of the experiment, a change in the nature of cortisol-binding characteristics of the intestine during AG treatment, or a requirement for one or more metabolic or hormonal intermediates not present in the in vitro system.

The glucocorticoid effect on ion transport was probably not secondary to morphological alterations, and, therefore, its delayed onset cannot be explained in this way. Our observations on histology are consistent with previous studies of the steroid-treated rat ileum that failed to demonstrate major changes in villus height or

villus:crypt ratio (2, 48). Although there is no evidence for changes in static morphology, cell turnover, and mitotic rates have been reported to increase in steroid-treated rat ileum (48) and to decrease in adrenalectomized rat jejunum (49), suggesting that the steroid-treated intestine has a greater proportion of younger cells. Whether such a change could be a significant factor in the enhancement of Na absorption by MP is unknown.

Glucocorticoids may act either directly by altering the synthesis of a specific protein or indirectly by altering the regulatory effects of other neurohumoral transmitters and hormones. Glucocorticoid-induced changes in mRNA and protein metabolism (50) may lead to changes in membrane ionic permeabilities or pump activities. Alternately, glucocorticoids may be important in the regulation of prostaglandin-prostacycline pathways in the intestine because suppression of prostaglandin synthesis by glucocorticoids has been noted in other tissues (51, 52). Prostaglandins have been shown to stimulate secretion in the intestine (41, 53), and therefore inhibition of their synthesis could result in increased absorption of Na and Cl.

Both mineralocorticoids and glucocorticoids are absorptive stimuli, but whether they act through similar or separate mechanisms is yet to be determined. Aldosterone is clearly associated with increased Na absorption in the colon, but its role in the small bowel is unclear. Although specific mineralocorticoid receptors have been demonstrated in rat ileum (54), pharmacologic doses of a mineralocorticoid had no apparent effect on fluid transport in rat ileum (2). The effect of physiologic amounts of mineralocorticoid on small bowel fluid transport has not been determined. The glucocorticoid effect on the colon is inhibited by spironolactone (40) and, therefore, has not been clearly differentiated from a mineralocorticoid effect.

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