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Research Article

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Prevention and Reversal of Cholera Enterotoxin-Induced Intestinal Secretion by Methylprednisolone Induction of Na^+-K^+-ATPase

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ABSTRACT The relationship of the mucosal enzyme systems Na^+-K^+-activated adenosine triphophatase (Na-K-ATPase) and adenylate cyclase and their associated intestinal transport processes was studied in the rat ileum. Two ileal loops were constructed in each anesthetized rat; one loop was inoculated with saline, the other loop with choleragen. Net transport of water and electrolytes was measured in vivo after which enzyme activity was measured in the mucosa of the perfused loops. All doses of choleragen between 5 and 150 μg decreased water movement as early as 3½ h after inoculation. A linear relationship between the dose of choleragen and the level of net water and electrolyte secretion was observed when choleragen doses between 5 and 150 μg were incubated in ileal loops for 4 h. Adenylate cyclase activity was always increased in secreting intestinal loops, whereas Na-K-ATPase was unaffected by choleragen. In animals pretreated with methylprednisolone acetate, 3 mg/100 g per day for 3 days before loop inoculation, saline loops had enhanced mucosal Na-K-ATPase activity and increased net water and electrolyte absorption; choleragen-exposed loops had increased adenylate cyclase and Na-K-ATPase activities, and net absorption of water and electrolytes 4 h after inoculation. These effects of methylprednisolone acetate were still present 19½ h after inoculation. When a single injection of methylprednisolone acetate was given 3½ h after choleragen inoculation, both adenylate cyclase and Na-K-ATPase were activated, and net intestinal absorption of water and electrolytes was observed 19½ h after inoculation. These results suggest that methylprednisolone can prevent and reverse the secretory effects of choleragen by selectively stimulating a coexisting absorptive process.

INTRODUCTION

A physiologic role for the mucosal enzyme Na^+-K^+-activated adenosine triphosphatase (Na-K-ATPase) [1] in intestinal electrolyte absorption has been proposed (1, 2). However, the relationship between the action of this enzyme and other intestinal enzyme systems believed to be involved in the transport of water and electrolytes (3, 4) has not been defined. One way of evaluating this relationship would involve the selective alteration and simultaneous measurement of these enzymes and their associated transport processes in the same segment of intestinal mucosa. It is well recognized that the intraluminal inoculation of cholera enterotoxin activates the intestinal adenylate cyclase-cyclic 3′5′ monophosphate (cAMP) enzyme system and produces net secretion of water and electrolytes (3–5). The recent finding that the parenteral administration of the glucocorticoid methylprednisolone acetate specifically enhances mucosal Na-K-ATPase activity and increases intestinal absorption of sodium and water (2) was significant in this regard since animals could now be exposed to agents known to stimulate intestinal absorption and secretion simultaneously. Our development of a reproducible model for cholera enterotoxin action in the rat ileum

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[1] *Abbreviations used in this paper:* Na-K-ATPase, Na^+-K^+-activated adenosine triphosphatase; PEG, polyethylene glycol.

allowed us to (*a*) study the relationship of these enzyme and transport systems and (*b*) elucidate the mechanisms by which glucocorticoids inhibit cholera enterotoxin-induced intestinal secretion, an observation originally reported by Jacoby and Marshall (6).

METHODS

Normal male albino Walter Reed rats[2] weighing 250–350 g were maintained on a standard rat chow diet with free access to water. Each animal was anesthetized with sodium pentobarbital (6.5 mg/100 g), and two 15-cm ileal loops were constructed 5 cm apart beginning 40 cm proximal to the ileocecal valve. Each loop was inoculated with a total volume of 1.0 ml of either 0.9% saline or purified cholera enterotoxin dissolved in saline (choleragen) (lot 0972, prepared by R. A. Finkelstein, M.D. (7) and generously provided by C. E. Miller, D.V.M. from the National Institutes of Health, Bethesda, Md.). The dose range of choleragen was 5–150 μg. After inoculation, the rats were allowed water for 2–18 h, after which the inoculum was washed out of each loop and intestinal transport studies were performed.

In one group of rats, an aqueous suspension of methylprednisolone acetate (Depo-Medrol, Upjohn Co., Kalamazoo, Mich.), 3 mg/100 g, was injected subcutaneously each day for the 3 days preceding inoculation. This dose and time-course of methylprednisolone administration was shown to affect intestinal transport and Na-K-ATPase activity in our previous studies (2). In another group of rats, a single injection of methylprednisolone acetate, 3 mg/100 g, was given 3½ h after choleragen inoculation. A third group of rats was not treated with methylprednisolone.

Transport studies. Intestinal transport of electrolytes and water was measured by a modification of an in vivo perfusion technique previously described (8). After washing with 50 ml of warm saline, both ileal loops were cannulated and perfused at a constant temperature (37°C) and rate (0.5 ml/min) with a peristaltic pump (Harvard Apparatus Co., Inc., Millis, Mass., model 1203). Body temperature was maintained at 37°C with a thermocouple-controlled heating lamp. Perfusion consisted of a 60-min steady state followed by three 20-min collection periods. The perfusion solution was a balanced electrolyte solution consisting of 140 mM Na, 5.2 mM K, 119.8 mM Cl, 25 mM HCO_3, 1.2 mM Ca, 1.2 mM Mg, 2.4 mM HPO_4, 0.4 mM H_2PO_4, mannitol (to bring the final osmolality to 300 mosmol/l), and [^{14}C]polyethylene glycol (PEG) and unlabeled PEG (5 g/l) as a nonabsorbable marker. After the perfusion studies, the lengths of both ileal loops were measured in a uniform manner by one observer, and they were immediately prepared for enzyme analysis.

Net water and electrolyte transport was calculated as previously described (8, 9) and expressed as microliter or μeq/20 min per cm length of loop. Net absorption from the lumen was expressed as a positive value, net secretion into the lumen as a negative value. Mean values were obtained for each loop by averaging the results of the three collection periods. For each perfusion period, PEG recovery was determined, and periods in which PEG recovery was not 100±5% were discarded. [^{14}C]PEG activity was measured in a Beckman Liquid Scintillation System LS-345 (Beckman Instruments, Inc., Fullerton, Calif.). Quench corrections were made by the method of external standards. Na and K were measured by flame photometry (IL model 143, Instrumentation Laboratories, Lexington, Mass.), Cl by coulometric titration (Buchler Instruments, Inc., Fort Lee, N. J.), and osmolality by freezing point depression (Advanced Instruments, Inc. Osmometer, Needham Heights, Mass.). The difference between the net movement of Na plus K and Cl in each loop was called residual anion movement since in these experiments bicarbonate movement accounted for most of the residual ion movement ($J_{Na} + J_K - J_{Cl}$) (10).

Samples of all ileal segments were placed in 10% formalin, stained with hematoxylin and eosin and periodic acid Schiff, coded, and examined by one of the authors. In preliminary studies, no effect of methylprednisolone or the perfusion studies on intestinal histology was observed.

Enzyme assays. Mucosa was obtained from perfused ileal segments by scraping with a glass slide. One part of the mucosa was homogenized with a Teflon pestle and iced glass homogenizer in a solution containing 130 mM NaCl, 5 mM Na_2EDTA, 30 mM imidazole, and 2.4 mM sodium deoxycholate (pH 6.8). The membrane-rich pellet obtained after successive centrifugations at 770 *g* and 10,000 *g* for 10 min at 0°C (11) was assayed for Na-K-ATPase and Mg-activated ATPase activities as previously described (2, 12). Approximately 100 μg of pellet protein was incubated for 15 min at 37°C in a solution containing 100 mM NaCl, 20 mM KCl, 10 mM imidazole, 5.4 mM $MgCl_2$, and 5.4 mM disodium ATP (Grade II, Sigma Chemical Co., St. Louis, Mo.). The inorganic phosphate liberated was measured spectrophotometrically, and results were expressed as micromoles of inorganic phosphate liberated per milligram protein per hour.

Simultaneously, another part of the mucosal scrapings was homogenized with an iced sintered glass homogenizer in a solution containing 75 mM tris-(hydroxymethyl) aminomethane and 25 mM $MgCl_2$ (pH 7.6). The whole homogenate was assayed for adenylate cyclase activity by the method of Krishna, et al. (13) with minor modifications as previously described (2). Approximately 50 μg of homogenate protein was incubated for 5 min at 37°C in a solution containing 1.5 mM ATP, 1 μCi (α-^{32}P) ATP (New England Nuclear, Boston, Mass.), 10 mM $MgCl_2$, 10 mM theophylline, 30 mM tris-(hydroxymethyl) aminomethane, 5 mM phospho (enol) pyruvate, 50 μg/ml pyruvate kinase, and 20 μg/ml myokinase. After passage over columns of BioRad (AG50W-X4) resin (BioRad Laboratories, Richmond, Calif.), and alumina, radioactive cAMP in the eluate was measured in a Beckman Liquid Scintillation System LS-345. Appropriate corrections were made for incubations run without enzyme and for the incomplete recovery of cAMP. Results were expressed as picomoles cAMP formed per milligram protein per 5 min. Protein concentrations were determined by the method of Lowry et al. (14).

In preliminary studies, no effect of the perfusion studies on ATPase or adenylate cyclase activity was noted, and whether choleragen was inoculated in the proximal or distal loop did not alter the effects of choleragen or methylprednisolone on enzyme activity or electrolyte transport.

Statistical analyses were performed by Student's *t* test for paired or unpaired data and were two tailed; linear regression analysis was by the method of least squares (15). All results are expressed as the mean±SE.

[2] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

TABLE I

*Effect of Choleragen Dose on Net Ileal Electrolyte Movement 4 h after Inoculation**

Choleragen dose	Loop inoculum	*n*	Electrolyte movement			
			Na	K	Cl	R
μg				*μeq/20 min per cm*		
	Saline	8	1.36±0.37	−0.04±0.03	1.88±0.36	−0.56±0.41
150	Choleragen	8	−2.63±0.64	−0.28±0.08	0.36±0.54	−3.19±0.53
			$P < 0.001$	$P < 0.02$	$P < 0.05$	$P < 0.001$
	Saline	4	1.60±0.59	−0.04±0.02	2.48±0.39	−0.65±0.38
75	Choleragen	4	−2.14±0.65	−0.21±0.03	0.77±0.39	−2.94±0.35
			$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.001$
	Saline	9	1.36±0.32	−0.03±0.01	2.51±0.22	−1.06±0.36
50	Choleragen	9	−1.25±0.41	−0.14±0.02	0.87±0.41	−2.25±0.31
			$P < 0.001$	$P < 0.001$	$P < 0.025$	$P < 0.05$
	Saline	5	1.71±0.38	−0.07±0.03	2.29±0.33	−0.66±0.55
25	Choleragen	5	−0.48±0.46	−0.18±0.04	1.33±0.38	−1.99±0.34
			$P < 0.005$	$P < 0.05$	NS	$P < 0.05$
	Saline	4	1.64±0.38	−0.02±0.03	1.98±0.41	−0.40±0.36
10	Choleragen	4	0.50±0.37	−0.09±0.08	2.29±0.60	−1.47±0.25
			$P < 0.05$	$P < 0.05$	NS	$P < 0.05$
	Saline	7	1.64±0.38	−0.01±0.02	1.88±0.46	−0.90±0.34
5	Choleragen	7	0.84±0.43	−0.06±0.02	2.01±0.39	−1.42±0.51
			NS	$P < 0.05$	NS	NS

* Results are expressed as the mean±SE. *n* indicates the number of animals studied. P values represent comparisons of saline and choleragen loops in individual animals (paired *t* test). R represents net residual anion movement ($J_{Na} + J_K - J_{Cl}$). A positive sign indicates net absorption from the lumen, a negative sign net secretion into the lumen.

RESULTS

Effect of choleragen on rat ileum. Inoculation of rat ileal loops with choleragen at doses between 5 and 150 μg reproducibly affected ileal transport of water and electrolytes and mucosal adenylate cyclase activity (Table I, Fig. 1). Water and electrolyte transport and enzyme activity in adjacent loops inoculated with 0.9% saline were unaffected. The levels of net water and electrolyte absorption observed in these saline-inoculated loops were similar to those previously reported from this laboratory (2). 4 h after choleragen inoculation, all doses of choleragen decreased net water and Na transport in comparison with adjacent ileal loops inoculated with saline. Net secretion of water and Na occurred at choleragen doses at or above 25 μg. Decreased net Cl absorption was seen in loops inoculated with 50 μg or more of choleragen. The minimal level of K secretion present in saline controls loops was significantly increased by all doses of choleragen between 5 and 150 μg. The residual anion movement, probably representing net secretion of bicarbonate (10), also was enhanced by all choleragen doses. These changes in net water and electrolyte transport were all found to be linearly related to the dose of choleragen. The absolute level of net water transport decreased progressively with the dose of choleragen over the 5–150 μg range ($r = 0.91$, slope = −0.08 μl/20 min per cm per μg, $P < 0.01$) (Fig. 1 middle panel). When the difference in net water movement between choleragen and saline-inoculated loops in each animal was plotted against choleragen dose, a linear relationship was again observed ($r = 0.98$, slope = −0.12, $P < 0.01$) (Fig. 1 lower panel). A similar linear relationship was found between choleragen dose and the absolute level of net Na movement ($r = 0.89$, slope = −0.02, $P < 0.01$), K movement ($r = 0.91$, slope = −0.001, $P < 0.02$), Cl movement ($r = 0.87$, slope = −0.01, $P < 0.05$) and residual anion movement ($r = 0.93$, slope = −0.01, $P < 0.01$); and between choleragen dose and the difference in the movement of each ion between choleragen and saline-inoculated loops (Na: $r = 0.90$, slope = −0.02, $P < 0.01$; K: $r = 0.98$, slope = −0.001, $P < 0.01$; Cl: $r = 0.71$, slope = −0.01, $P < 0.05$; residual anion: $r = 0.91$, slope = −0.01, $P < 0.01$).

4 h after inoculation, choleragen doses of 5, 50, and 150 μg increased ileal mucosal adenylate cyclase activity (Fig. 1), but did not affect the specific activities of

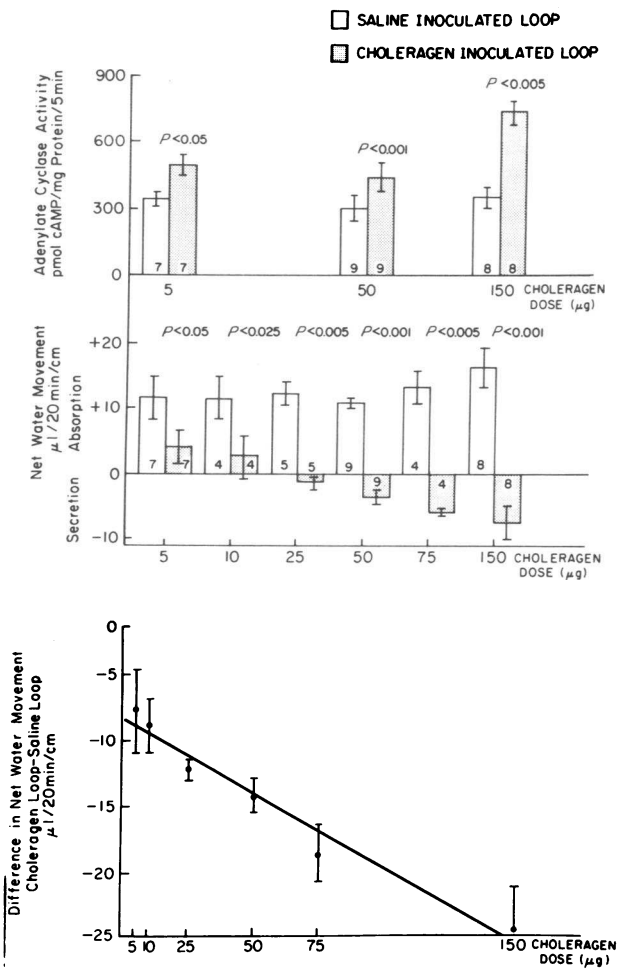

FIGURE 1 Effect of choleragen dose on ileal enzyme activity and net water movement 4 h after inoculation. 5, 50, and 150 μg of choleragen all significantly increased adenylate cyclase activity (upper panel). Increasing doses of choleragen had progressively greater effects on net water movement (middle panel) with net water secretion occurring at a choleragen dose of 25 μg. The relationship between the absolute level of net water movement and the dose of choleragen was linear over the entire dose range studied ($r = 0.91$, slope $= -0.08$ μl/20 min per cm/μg, $P < 0.01$), as was the relation between choleragen dose and the difference in net water movement between choleragen and saline-inoculated loops in each animal ($r = 0.98$, slope $= -0.12$, $P < 0.01$) (lower panel). Values are mean±SE. P values above bars represent comparisons of saline and choleragen inoculated loops in individual animals. Number in bar indicates the number of animals studied.

Na-K-ATPase or Mg-activated ATPase. Activation of adenylate cyclase was still present as late as 19½ h after choleragen inoculation. In fact, increases in adenylate cyclase activity accompanied the changes in water and electrolyte transport in every loop inoculated with choleragen in which both measurements were made. Although a linear correlation between the level of adenylate cyclase activity and the level of net water movement in these choleragen-inoculated loops was not demonstrated, activation of adenylate cyclase was greater in tissue exposed to 150 μg as compared to tissue exposed to 5 μg or 50 μg of choleragen.

The time-course of the choleragen-induced ileal secretion was also characterized. The level of net water secretion after inoculation of 50 μg of choleragen did not vary significantly when studied at 30-min intervals between 3½ and 7 h after inoculation (see 50 μg dose in Fig. 1 for 4 h value). 5 μg of choleragen also produced a constantly decreased level of net water absorption between 3½ and 7 h after inoculation (see 5 μg dose in Fig. 1 for 4 h value), although net secretion of water was not observed. However, 19½ h after inoculation, 5 μg of choleragen induced net secretion of water (−2.53±0.57 [8] vs. 5.45±0.84 [8] μl/20 min per cm, $P < 0.001$) in conjunction with a decrease in the absorption of Na (−0.01±0.34 [8] vs. 0.44±0.28 [8] μeq/20 min per cm) (Fig. 3 left panel, Table III, "Untreated animals").

Up to 5 h after choleragen inoculation, the histologic changes caused by all doses of choleragen between 5 and 150 μg consisted primarily of focal villus tip cell flattening. Ileal mucosa exposed to 5 μg of choleragen for 19½ h showed occasional villus tip cell extrusion and mild submucosal vascular congestion in addition to focal villus tip cell flattening. At 19½ h, choleragen doses above 5 μg produced progressively greater mucosal damage presumably by increasing the quantity of secreted fluid.

Prevention of choleragen-induced ileal secretion by methylprednisolone. To determine the effect of methylprednisolone pretreatment on choleragen-induced intestinal secretion, methylprednisolone acetate, 3 mg/100 g, was injected each day for 3 days before loop inoculation. In ileal loops studied 4 h after inoculation with saline, both mucosal Na-K-ATPase activity and water absorption were enhanced in steroid treated as compared to untreated rats (Fig. 2). Methylprednisolone pretreatment also enhanced Na and Cl absorption and K secretion, but did not affect residual anion secretion (Table II). The specific activities of adenylate cyclase and Mg-activated ATPase, were unaffected by steroid treatment. These findings have been reported previously by this laboratory (2).[3] 4 h after inoculation of 50 μg of choleragen in methylprednisolone treated rats, a rise in mucosal adenylate cyclase activity similar to the rise found in untreated animals was observed. However, in comparison with the net secretion of water and Na seen in untreated rats, methylprednisolone pretreatment in-

[3] Phosphodiesterase activity in rat ileal mucosa is also unaffected by methylprednisolone treatment. Donowitz, M., and A. N. Charney. Unpublished observations.

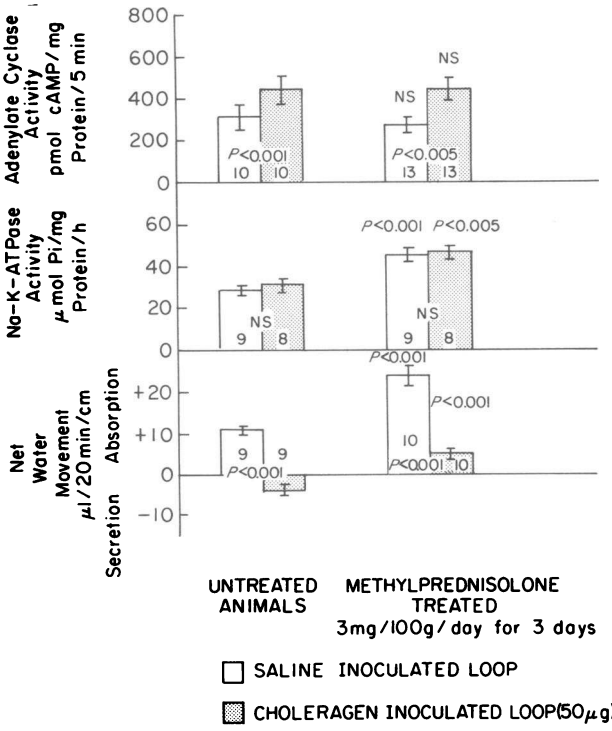

FIGURE 2 Effect of methylprednisolone pretreatment on ileal enzyme activity and net water movement 4 h after choleragen inoculation. Choleragen increased adenylate cyclase activity ($P < 0.001$) and caused net water secretion ($P < 0.001$) in untreated animals. In methylprednisolone treated as compared to untreated animals: saline inoculated loops had increased Na-K-ATPase activity ($P < 0.001$) and increased net water absorption ($P < 0.001$); choleragen inoculated loops had identically elevated adenylate cyclase levels and increased Na-K-ATPase activity ($P < 0.005$), and exhibited net water absorption ($P < 0.001$). Values are mean±SE. P values above bars represent comparisons of treated and untreated animals. P values in bars represent comparisons of saline and choleragen inoculated loops in individual animals. Number in bar indicates the number of animals studied.

creased mucosal Na-K-ATPase activity in these choleragen-exposed loops, and net absorption of water (Fig. 2) and Na (0.23±0.33 [10] vs. −1.25±0.41 [9] μeq/20 min per cm, $P < 0.02$) was observed. Cl absorption was greater and residual anion secretion was smaller in these choleragen-inoculated loops in methylprednisolone treated animals as compared to untreated animals, although these changes did not reach statistical significance (Table II).

Of particular interest was the finding that the decrements in ileal water, Na, K, Cl, and residual anion movement produced by a 50 μg dose of choleragen 4 h after inoculation were very similar in untreated and methylprednisolone treated animals (Fig. 2, Table II). Thus, the difference in water movement (−14.28±1.34 μl/20 min per cm) between choleragen (−3.57±1.03 [9]) and saline-inoculated loops (10.71±0.70 [9]) in untreated animals was similar to the difference in water movement (−19.13±2.82 μl/20 min per cm) between choleragen (4.46±0.99 [10]) and saline-inoculated loops (23.59±2.15 [10]) in methylprednisolone treated animals. The effect of choleragen on electrolyte movement (the difference between choleragen and saline-inoculated loops) in untreated and methylprednisolone treated animals was likewise remarkably similar (Na: −2.61±0.33 [9] vs. −2.36±0.30 [10]; K: −0.12±0.02 [9] vs. −0.12 ±0.02 [10]; Cl: −1.64±0.53 [9] vs. −2.17±0.58 [10]; residual anions: −1.19±0.48 [9] vs. −0.30±0.41 [10] μeq/20 min per cm). Apparently the effect of choleragen on ileal water and electrolyte movement, as on adenylate cyclase activity (Fig. 2), is unaffected by methylpredniso-

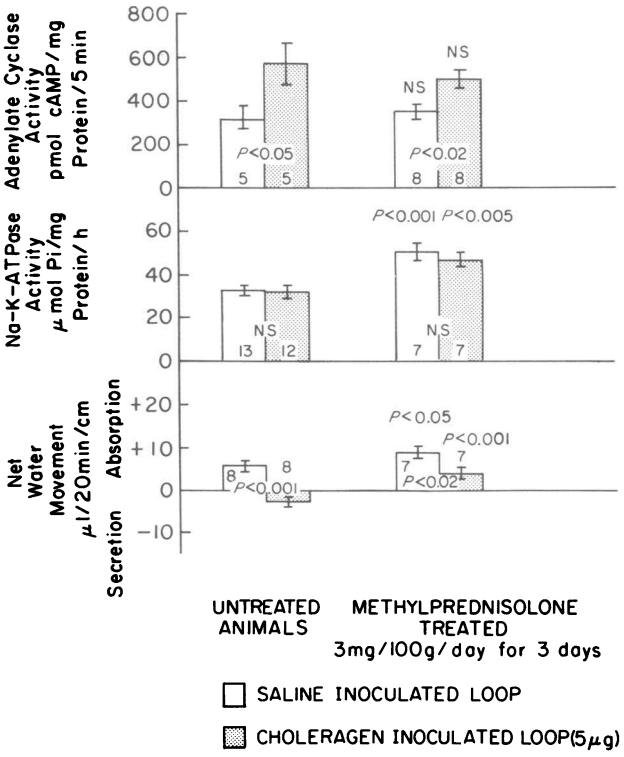

FIGURE 3 Effect of methylprednisolone pretreatment on ileal enzyme activity and net water movement 19½ h after choleragen inoculation. Choleragen increased adenylate cyclase activity ($P < 0.05$) and caused net water secretion ($P < 0.001$) in untreated animals. In methylprednisolone treated as compared to untreated animals: saline inoculated loops had increased Na-K-ATPase activity ($P < 0.001$) and increased net water absorption ($P < 0.05$); choleragen inoculated loops had identically elevated adenylate cyclase levels and increased Na-K-ATPase activity ($P < 0.005$), and exhibited net water absorption ($P < 0.001$). Values are mean±SE. P values above bars represent comparisons of treated and untreated animals. P values in bars represent comparisons of saline and choleragen inoculated loops in individual animals. Number in bar indicates the number of animals studied.

TABLE II

*Effect of Methylprednisolone Pretreatment on Net Ileal Electrolyte Movement 4 h after Choleragen Inoculation**

	n	Electrolyte movement			R
		Na	K	Cl	
		μeq/20 min per cm			
Untreated animals					
Saline loop	9	1.36±0.32	−0.03±0.01	2.51±0.22	−1.06±0.36
Choleragen loop (50 μg)	9	−1.25±0.41	−0.14±0.02	0.87±0.41	−2.25±0.31
vs. Saline loop (untreated)		$P < 0.001$	$P < 0.001$	$P < 0.025$	$P < 0.05$
Methylprednisolone treated (3 mg/100 g per day for 3 days before inoculation)					
Saline loop	10	2.59±0.21	−0.10±0.02	3.35±0.38	−0.86±0.29
vs. Saline loop (untreated)		$P < 0.01$	$P < 0.05$	$P < 0.02$	NS
Choleragen loop (50 μg)	10	0.23±0.33	−0.21±0.02	1.18±0.28	−1.16±0.25
vs. Choleragen loop (untreated)		$P < 0.02$	$P < 0.025$	NS	NS
vs. Saline loop (Methylprednisolone, 3 days)		$P < 0.001$	$P < 0.001$	$P < 0.005$	NS

* Results are expressed as the mean±SE. *n* indicates the number of animals studied. P values represent comparisons of saline and choleragen loops in individual animals (paried *t* test) and comparisons of methylprednisolone treated and untreated animals (unpaired *t* test). R represents net residual anion movement ($J_{Na} + J_K - J_{Cl}$). A positive sign indicates net absorption from the lumen, a negative sign net secretion into the lumen.

lone pretreatment and is independent of the initial level of net water and electrolyte movement.

To determine whether methylprednisolone pretreatment continued to prevent choleragen-induced ileal secretion beyond 4 h after inoculation, the effect of methylprednisolone 3 mg/100 g per day for 3 days, was examined in toxin-exposed loops 19½ h after inoculation. A 5-μg inoculum of choleragen was chosen to avoid major histologic alterations (vide supra). However, the extended time period after loop inoculation did result in lower levels of water and Na absorption in the saline inoculated loops in untreated animals as compared to similar loops studied 4 h after inoculation (compare Fig. 3 and Table III with Fig. 1 and Table I). Nevertheless, the 5-μg dose of choleragen produced net secretion of water, and elevated levels of adenylate cyclase 19½ h after inoculation in untreated animals (Fig. 3). Decreased absorption of Na and Cl and increased secretion of residual anions were also noted, although these changes were not statistically significant (Table III).

TABLE III

*Effect of Methylprednisolone Pretreatment on Net Ileal Electrolyte Movement 19½ h after Choleragen Inoculation**

	n	Electrolyte movement			R
		Na	K	Cl	
		μeq/20 min per cm			
Untreated animals					
Saline loop	8	0.44±0.28	−0.08±0.03	1.38±0.33	−1.02±0.50
Choleragen loop (5 μg)	8	−0.01±0.34	−0.10±0.03	1.19±0.32	−1.30±0.46
vs. Saline loop (untreated)		NS	NS	NS	NS
Methylprednisolone treated (3 mg/100 g per day for 3 days before inoculation)					
Saline loop	7	1.17±0.19	−0.13±0.06	2.53±0.29	−1.49±0.33
vs. Saline loop (untreated)		$P < 0.05$	NS	$P < 0.025$	NS
Choleragen loop (5 μg)	7	0.65±0.47	−0.12±0.04	2.45±0.42	−1.92±0.23
vs. Choleragen loop (untreated)		$P < 0.02$	NS	$P < 0.05$	NS
vs. Saline loop (methylprednisolone, 3 days)		NS	NS	NS	NS

* Results are expressed as the mean±SE. *n* indicates the number of animals studied. P values represent comparisons of saline and choleragen loops in individual animals (paired *t* test) and comparisons of methylprednisolone treated and untreated animals (unpaired *t* test). R represents net residual anion movement ($J_{Na} + J_K - J_{Cl}$). A positive sign indicates net absorption from the lumen, a negative sign net secretion into the lumen.

TABLE IV

*Effect of Methylprednisolone Administered 3½ h after Choleragen Inoculation on Net Ileal Electrolyte Movement**

	n	Electrolyte Movement			
		Na	K	Cl	R
			μeq/20 min per cm		
Untreated animals					
Saline loop	8	0.44±0.28	−0.08±0.03	1.38±0.33	−1.02±0.50
Choleragen loop (5 μg)	8	−0.01±0.34	−0.10±0.03	1.19±0.32	−1.30±0.46
vs. Saline loop (untreated)		NS	NS	NS	NS
Methylprednisolone treated (3 mg/100 g 3½ h after inoculation)					
Saline loop	7	1.50±0.31	−0.04±0.06	2.41±0.56	−0.95±0.61
vs. Saline loop (untreated)		$P < 0.025$	NS	NS	NS
Choleragen loop (5 μg)	7	0.54±0.27	−0.10±0.03	1.44±0.68	−1.00±0.59
vs. Choleragen loop (untreated)		NS	NS	NS	NS
vs. Saline loop (methylprednisolone, 16 h)		NS	NS	NS	NS

* All measurements were made 19½ h after choleragen inoculation, 16 h after a single methylprednisolone injection. Results are expressed as the mean±SE. *n* indicates the number of animals studied. P values represent comparisons of saline and choleragen loops in individual animals (paired *t* test) and comparisons of methylprednisolone treated and untreated animals (unpaired *t* test). R represents net residual anion movement ($J_{Na} + J_K - J_{Cl}$). A positive sign indicates net absorption from the lumen, a negative sign net secretion into the lumen.

In methylprednisolone treated rats, saline-inoculated loops had enhanced mucosal Na-K-ATPase activity, and increased net water, Na and Cl absorption (Fig. 3, Table III). Choleragen-exposed loops in these methylprednisolone treated animals had increased adenylate cyclase and Na-K-ATPase activities and exhibited net water absorption (Fig. 3). Net Na absorption and increased Cl absorption accompanied net water absorption in these choleragen-inoculated loops.

Reversal of choleragen-induced ileal secretion by methylprednisolone. While methylprednisolone clearly could prevent choleragen-induced intestinal secretion, it was also of interest to determine whether methylprednisolone could reverse this secretion once it had been initiated. In untreated animals, a significant decrease in ileal net water absorption (2.06±1.32 [6] vs. 8.27±1.92 [7] μl/20 min per cm, $P < 0.01$) was already present 3½ h after exposure to 5 μg of choleragen as was an increase in residual anion secretion (−1.60±0.53 [6] vs. −0.80±0.28 [7], $P < 0.05$) and K secretion (−0.06± 0.20 [6] vs. −0.01±0.20 [7], $P < 0.05$). Furthermore, activation of adenylate cyclase and net secretion of water and Na were still apparent in the ileal loops of these untreated animals 19½ h after inoculation (Fig. 4, Table IV). Since an increase in ileal Na-K-ATPase activity was apparent as early as 16 h after a single injection of methylprednisolone, 3 mg/100 g, (Fig. 4), a single 3 mg/100 g dose of methylprednisolone was injected 3½ h after inoculation of 5 μg of choleragen, and ileal transport and enzyme activity were measured 19½ h after inoculation. As shown in Fig. 4 and Table IV, methylprednisolone reversed choleragen-induced ileal secretion of water, increased the net absorption of Na and Cl and reduced residual anion secretion. The change in water movement to net water absorption was highly significant ($P < 0.005$), and although the changes in electrolyte movement did not reach statistical significance, they mirrored the changes found 4 h (Table II) and 19½ h (Table III) after choleragen inoculation in animals pretreated with methylprednisolone. In addition, both Na-K-ATPase and adenylate cyclase activities were increased at the time these measurements were made, 16 h after the methylprednisolone injection. In fact, neither activation of Na-K-ATPase nor reversal of choleragen-induced ileal secretion was observed earlier than 16 h after methylprednisolone treatment. The ability of methylprednisolone to overcome the effects of choleragen, thus, coincides with its enhancement of mucosal Na-K-ATPase activity, and is unaffected by prior activation of adenylate cyclase and the secretory process by choleragen.

DISCUSSION

These studies examined the effects of the glucocorticoid methylprednisolone on normal intestinal water and electrolyte transport and on choleragen-induced intestinal secretion. The increased ileal absorption of sodium and water after methylprednisolone administration, and the close association between these transport changes and the selective activation of Na-K-ATPase reported previously (2), were confirmed here. We also demonstrated that this glucocorticoid can modify the intestinal secretion induced by choleragen. Methylprednisolone

was able (*a*) when given before choleragen inoculation, to prevent choleragen from inducing intestinal secretion, and (*b*) when given after choleragen inoculation, to overcome the secretory effects of this enterotoxin. Since the transport changes induced by choleragen were accompanied and presumably mediated by activation of the single enzyme system adenylate cyclase (3–5, 16, 17), the relationship between the Na-K-ATPase and adenylate cyclase enzyme system was also elucidated.

The use of paired ileal loops provided a convenient way to study the in vivo effects of both methylprednisolone and choleragen on intestinal transport and enzyme activity simultaneously. Differences in the levels of water and electrolyte transport and enzyme activity between two adjacent loops in an individual animal could be attributed to choleragen, whereas differences in similarly inoculated loops between methylprednisolone treated and untreated animals could be confidently attributed to methylprednisolone. The ileum was studied because in preliminary experiments the effect of choleragen on jejunal water and electrolyte transport was less reproducible, and because in the rat, methylprednisolone exerts its maximum effect on water and electrolyte absorption in the ileum (2). Methylprednisolone treatment enhanced Na-K-ATPase activity and net sodium, chloride and water absorption without significantly changing residual anion movement in saline inoculated loops as early as 16 h after a single injection; these changes were still present 30 h after the last of three daily injections. In untreated animals, the loop inoculated with choleragen exhibited adenylate cyclase activation and net sodium and water secretion coincident with normal adenylate cyclase activity and sodium and water absorption in the adjacent saline-inoculated loop. The lack of an effect on mucosal Na-K-ATPase activity by choleragen, previously reported in the rabbit (5), was also found in these studies in the rat. In addition to causing net sodium and water secretion, choleragen decreased the net absorption of chloride, and increased net residual anion (bicarbonate) secretion. The failure of choleragen to cause net chloride secretion was also reported in another in vivo cholera model (in the dog) (18), and contrasts with choleragen-induced net secretion of chloride observed in vitro (3, 19, 20). The in vivo findings are probably secondary to a change in potential difference: the increased potential difference (lumen negative) produced by choleragen resulted in increased passive chloride movement along the electrical gradient which masked the choleragen-induced active chloride secretion (3, 19).

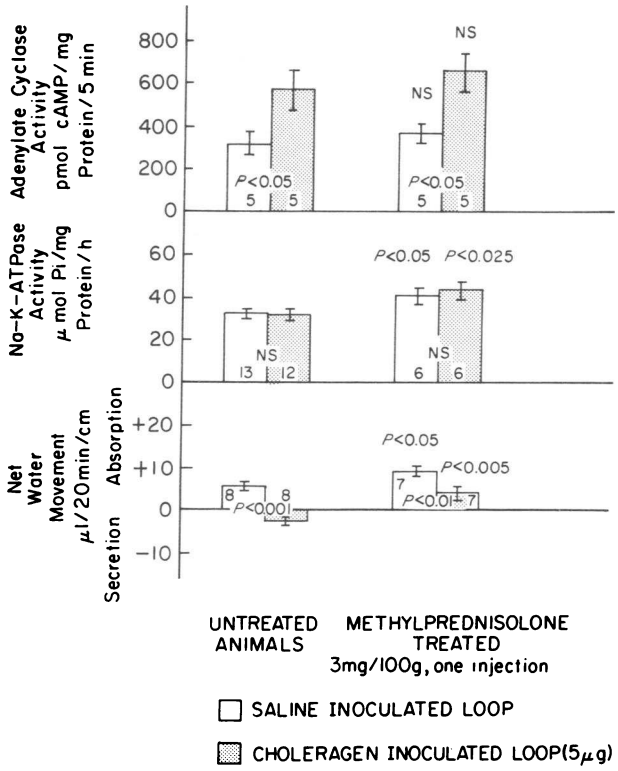

FIGURE 4 Effect of methylprednisolone injected 3½ h after choleragen inoculation on ileal enzyme activity and net water movement. All measurements were made 19½ h after inoculation. Choleragen increased adenylate cyclase activity ($P < 0.05$) and caused net water secretion ($P < 0.001$) in untreated animals. In methylprednisolone treated as compared to untreated animals: saline inoculated loops had increased Na-K-ATPase activity ($P < 0.05$) and increased net water absorption ($P < 0.05$); choleragen inoculated loops had identically elevated adenylate cyclase levels and increased Na-K-ATPase activity ($P < 0.025$), and exhibited net water absorption ($P < 0.005$). Values are mean±SE. P values above bars represent comparisons of treated and untreated animals. P values in bars represent comparisons of saline and choleragen inoculated loops in individual animals. Number in bar indicates the number of animals studied.

The effect of choleragen on ileal water and electrolyte transport was dose-dependent between 5 and 150 μg. In fact, a linear relationship was found between the dose of choleragen and the absolute levels of water, sodium, potassium, chloride, and bicarbonate movement, as well as between the dose of choleragen and the change from saline control values in the levels of water, sodium, potassium, chloride, and bicarbonate movement. The effect of choleragen on ileal water transport, in addition, could be demonstrated as early as 3½ h, and as late as 19½ h after inoculation. Significant histological alterations did not accompany ileal water secretion 5 h after inoculation of up to 150 μg of choleragen. However, 19½ h after inoculation, only a dose as low as 5 μg of choleragen resulted in water secretion and only minor histological alterations, i.e, focal villus tip epithelial cell flattening and extrusion, and minimal submucosal vascular congestion. These minor histological changes,

nevertheless, quite possibly accounted for the quantitatively lesser effect of this 5 μg choleragen dose on electrolyte transport at 19½ h than at 4 h after inoculation (compare Tables I and III).

The prevention and reversal of choleragen-induced intestinal secretion by methylprednisolone were characterized by apparently independent alterations in (*a*) the processes of intestinal absorption and secretion, and (b) the Na-K-ATPase and adenylate cyclase enzyme systems which presumably mediate these transport processes. The activation of adenylate cyclase by choleragen was not affected by pretreatment with methylprednisolone (or by the rise in Na-K-ATPase activity). In addition, methylprednisolone pretreatment did not affect the magnitude of the secretory effect of choleragen on water and electrolyte movement. Thus, the difference in water or sodium movement between choleragen and saline-inoculated loops in untreated animals (water: −14.28±1.34 μl/20 min per cm; Na: −2.61±0.33 μeq/20 min per cm) was similar to the difference in water or sodium movement between choleragen and saline-inoculated loops in methylprednisolone treated animals (water: −19.13± 2.82 μl/20 min per cm; Na: −2.36±0.30 μeq/20 min per cm). These data indicate that the effect of choleragen on ileal water and electrolyte transport was still apparent although net secretion was prevented by prior enhancement of Na-K-ATPase activity and the absorptive process by methylprednisolone. Similarly, the effects of methylprednisolone pretreatment on Na-K-ATPase and water and electrolyte movement were equally apparent in choleragen and saline-inoculated ileal loops. More importantly, these effects were apparent even when methylprednisolone was administered after the choleragen-induced secretory process had been initiated. These findings suggest that the prevention and reversal of choleragen-induced intestinal secretion by methylprednisolone reflect a balance between two independent and oppositely-directed transport processes. Accordingly, choleragen inoculation and parenteral methylprednisolone administration simply represent highly selective means of altering each of these processes. We are suggesting, then, that either the absorptive or the secretory process can predominate, depending upon, among other factors, the relative levels of Na-K-ATPase-associated intestinal absorption, and adenylate cyclase-associated intestinal secretion.

In addition to the close association between glucose independent intestinal sodium absorption and the level of mucosal Na-K-ATPase activity demonstrated previously (*2*) and confirmed here, there is evidence to suggest that the Na-K-ATPase enzyme system may play a role in glucose-dependent sodium transport (1). Although the specific activity of Na-K-ATPase is unaffected by the presence of luminal glucose (*2*), enhancement of intestinal glucose absorption and glucose-dependent sodium absorption by methylprednisolone in concert with increases in Na-K-ATPase activity was recently described (*2*). Furthermore, ouabain, in serosal surface concentrations known to inhibit Na-K-ATPase activity (21), decreased the mucosal-to-serosal flux of 3-*O*-methylglucose in the in vitro studies of Czaky and Hara (*22*) and prevented the increase in short circuit current which normally follows the addition of actively transported sugars (1). Thus, sodium exit across the serosal surface of the intestinal epithelial cell may require Na-K-ATPase regardless of the mode of sodium entry across the luminal membrane (1). Our finding that the Na-K-ATPase enzyme system was unaffected by and acted independently of choleragen-induced intestinal secretion, then, may be relevant to several previous studies which showed that intraluminal glucose can overcome ongoing cholera enterotoxin-induced intestinal secretion both in vivo (*23–25*) and in vitro (19). These studies have been interpreted as indicating that glucose-dependent sodium absorption is intact during cholera enterotoxin-induced secretion. This interpretation was based, in part, on the finding that enhancement of sodium and water absorption by glucose was equally great in control and cholera enterotoxin-exposed intestinal mucosa (*23*). Our finding that intestinal Na-K-ATPase is functionally intact in choleragen-exposed mucosa also supports this interpretation. Apparently, an intact intestinal Na-K-ATPase enzyme system is the basis for the integrity of both glucose-dependent sodium absorption and methylprednisolone-stimulated glucose independent sodium absorption during cholera enterotoxin-induced intestinal secretion.

To the extent that the effects of methylprednisolone described here in rats can be extrapolated to humans, the mode of action of methylprednisolone in the treatment of diarrheal disease must be reexamined. An effect of glucocorticoids on human intestinal transport has been demonstrated indirectly (*26–28*), although the efficacy, minimal dose required, and time—course of action have not been determined. Moreover, the ability of methylprednisolone to increase glucose absorption and glucose-dependent as well as glucose independent intestinal Na absorption suggests that it may have even greater effects under optimal clinical conditions than we could demonstrate in rats in the absence of luminal glucose. Not withstanding these clinical implications, we believe the interactions of methylprednisolone and cholera enterotoxin described here will provide other insights into the mechanisms of intestinal electrolyte transport.

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