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Mucosal ion transport in acute viral enteritis

H. J. McCLUNG, M.D., D. G. BUTLER, C.V.M., PH.D., B. KERZNER, M.B.B.CH., F.C.P., D. G. GALL, M.D., F.R.C.P.(C), AND J. R. HAMILTON, M.D., F.R.C.P.(C)

Research Institute, The Hospital for Sick Children, Division of Gastroenterology, Department of Paediatrics, University of Toronto, Toronto, and Department of Clinical Studies, University of Guelph, Guelph, Ontario, Canada

Ion transport in the jejunal mucosa of 14- to 16-day-old piglets with severe diarrhea 40 hr after infection with transmissible gastroenteritis (TGE) virus was studied. In infected pigs Na^+ transport failed to respond normally to glucose when studied either in Ussing short-circuited chambers or in suspensions of enterocytes isolated selectively from jejunal villi. Theophylline, 10 mM, added to the chambers produced the same brisk electrical responses and increments in net Cl^- secretion in tissue from both infected and control groups. A defect in glucose-stimulated Na^+ absorption in the acute stage of a viral enteritis has been identified which probably contributes to the impaired lumen-to-extracellular fluid flux of Na^+ found previously in the jejunum of intact TGE-infected pigs. The mechanisms causing diarrhea in this invasive viral enteritis differ from those causing toxigenic diarrhea.

Transmissible gastroenteritis (TGE) is a specific corona virus infection of the upper intestinal epithelium of young pigs.[1-4] Previous experiments in 2- to 3-week-old pigs infected with TGE virus have established a consistent experimental model of an acute diarrheal illness in which disturbed electrolyte transport is a major factor contributing to diahrrea.[5] By means of an in vivo marker perfusion technique, abnormalities of Na^+ transport in the jejunum of infected animals were identified 40 hr after they were infected experimentally.[6] In the experiments to be described in vitro techniques were used at the same stage of the disease to characterize in more detail the epithelial transport defects that contribute to this viral diarrhea.

Material and Methods

Fifty 14- to 16-day-old pigs from eight litters of conventional York breed swine were studied. As in previous experiments, the animals to be infected received an oral dose of 2 ml of a suspension of Purdue strain TGE virus prepared as a bacteria-free homogenate of whole intestinal mucosa.[7] Infected pigs were isolated in special facilities. Controls were age-matched uninfected litter-mates. All animals were killed with 250 mg of sodium pentobarbital injected into the heart 40 hr after oral infection, by which time infected pigs all had diarrhea. Starting 20 cm distal to the ligament of Treitz, two adjacent segments of jejunum were quickly removed for flux studies, a 10-cm segment was removed for study in short-circuited Ussing chambers, and the next 30-cm segment was removed, from which villous enterocytes were isolated for subsequent measurement of Na^+ efflux rate constants.

For the short-circuited chamber studies a sheet of intestinal mucosa was dissected with ease from the underlying muscular layers and mounted on conventional conical leucite Ussing chambers which allowed a mucosal surface exposure of 1.29 cm^2. Each side of the tissue was bathed separately by 9 to 15 ml of buffer which was circulated by 95% O_2 and 5% CO_2 and kept at 37°C. The Krebs-bicarbonate-phosphate buffer contained Na^+ (140 mM), K^+ (10 mM), Mg^{++} (1.1 mM), Ca^{++} (1.25 mM), Cl^- (127.5 mM), HCO_3^- (25 mM), and $H_2PO_4^-$ (2 mM). Buffer *p*H, when aerated at 37°C with 95% O_2 and 5% CO_2, was stable, 7.4 ± 0.1. The spontaneous electrical potential difference (PD) produced by the tissue was measured (model 600B Voltmeter, Keithly Instruments, Cleveland, Ohio) and nullified (short-circuited) by an external source (Variable DC microamp source, Heathkit Instruments, Mississauga, Canada). $^{22}Na^+$ and $^{36}Cl^-$ obtained as a saline preparation were diluted to 10 μc each per ml, 1 ml of which was added to one side of the tissue; $^{24}Na^+$ was prepared locally by neutron activation (Slowpoke Reactor, Department of Medical Engineering and Applied Chemistry, University of Toronto, Canada) and added to the other side.

Stripped mucosa from each animal was divided into equal segments which were mounted on four individual chambers for

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Address requests for reprints to: Dr. J. R. Hamilton, Chief, Division of Gastroenterology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.

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Dr. McClung's present address is: Pediatric Gastroenterology Children's Hospital, 561 17th Street, Columbus, Ohio 43205.

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study in three consecutive periods; the first two periods lasted 45 min each, the last 55 min. All tissues were studied under basal conditions during the first period and in the presence of 30mM glucose added to both sides of the tissue during the second period. For the third period, one-half of the segments were studied after adding 1 mM ouabain to both sides and one-half after adding 10 mM theophylline to both sides. Tissue was allowed to equilibrate for the first 15 min of each period, except in the case of ouabain, when a 25-min period was allowed to permit the PD to fall to zero. One-milliliter samples were withdrawn from the bathing solutions at 10-min intervals for counting. The flux characteristics of the tissue were stable for at least 3 hr under the conditions of this experiment. $^{24}Na^+$ was counted in a well-type crystal gamma counter (Biogamma, Beckman Instruments, Fullerton, Calif.) with the threshold set to completely exclude the weaker radiations from $^{22}Na^+$. Three weeks later when $^{24}Na^+$ activity had decayed, $^{22}Na^+$ was counted in the same gamma counter, and the combined activities of $^{22}Na^+$ and $^{36}Cl^-$ were counted in a well-type beta scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Standards containing known amounts of $^{22}Na^+$ and $^{36}Cl^-$, singly and in combination, were used so that the two isotopes could be separated by calculating differential efficiencies of the beta and gamma counters. The PD of the tissue was measured at 10-min intervals; the current necessary to short-circuit the tissue was manually applied from an adjustable current source. In all experiments correction was made for the drop in electrical potential between bridge tips and tissue surfaces by determining any electrode asymmetry, system potentials, and buffer fluid resistance between electrode tips before mounting the tissues. The short-circuit current (I_{sc}) necessary to neutralize the PD of the tissue was calculated according to the graph method of Clarkson and Toole[8] except if the current exceeded 200 μa, when the method of Asano,[9] which gave comparable results, was used. Tissue conductance was calculated as the reciprocal of the tissue resistance and expressed as millisiemens per square centimeter. To determine unidirectional Na^+ and Cl^- fluxes, the steady-state rates of radioisotope transfer were divided by the specific activities of the initially labeled sides and by the surface area of the exposed tissue (1.29 cm^2).[10]

$$J_{ms}^{Na^+} = \frac{V_s(P_s^2 - P_s^1)}{\Delta t P_m^* A}$$

J_{ms}^{Na} = flux of Na^+ from mucosa to serosa in μEq $cm^{-2}hr^{-1}$;
V_s = volume of medium perfusing the serosal surface (in milliliters);
P_s^2 = counts per minute per milliliter in the serosal reservoir at time 2;
P_s^1 = counts per minute per milliliter in the serosal reservoir at time 1;
Δt = time interval between samples 1 and 2 (in hours);
P_m^* = specific activity of Na^+ isotope in the mucosal solution (in counts per minute per microequivalent);
A = area of mucosal membrane (1.29 cm^2).

In suspensions of epithelial cells, selectively isolated from villi by a vibration technique which excluded crypt cells, Na^+ efflux was measured using a technique described previously for rat cells except that the isolation solution and $MgCl_2$ wash solution contained 5 mM Tris-Tris HCl buffer instead of 2 mM Tris buffer.[11] In a previous preliminary experiment using isolated pig enterocytes, the technique used differed in that epithelial cells were isolated at 4°C, rather than 37°C, and sucrose K_2HPO_4 and albumin were not included in the isolation solution.[6] The harvested epithelial cells were loaded with radioactive Na^+ by suspending them in incubation medium containing $^{22}Na^+$. After being washed, the cells were resuspended in tracer-free medium and the Na^+ efflux rate constant was ascertained by measuring the rate of appearance of radioactive Na^+ in the medium. When glucose or ouabain was used it was added to the incubation medium during both loading and efflux periods. Na efflux was studied under basal conditions, as in previous experiments,[6 11] by substitution of equimolar mannitol for glucose.

Increments occurring in individual tissues were assessed with paired *t*-tests and control and infected tissues were compared using Student's *t*-tests. All variance is expressed as the standard error of the mean (SE). Flux data from the short-circuit chamber studies are expressed as μEq $cm^{-2}hr^{-1}$. All unidirectional fluxes are expressed as positive numbers; net flux is designated as positive (+) when in the mucosa to serosa (M→S, absorptive) direction and negative (−) when in the serosa to mucosa (S→M, secretory) direction. Na^+ efflux rate constants are reported as the amount of Na^+ exchanged per hour ($^0K_{Na^+}hr^{-1}$).

Results

Na^+ flux (table 1). Under basal conditions, that is, in the absence of glucose, unidirectional fluxes in control and infected tissue did not differ; net fluxes were secretory and identical. Control tissue responded to glucose as expected with a significant increase in M→S flux ($P < 0.001$) and a change of net flux to absorption ($P < 0.001$). In TGE tissue, M→S Na^+ flux failed completely to respond to glucose, and although there was a significant increment in net flux ($P < 0.005$), it was significantly less than in control tissue ($P < 0.001$). Ouabain added after glucose caused a significant reduction in net Na^+ flux in controls, ($P < 0.01$) but no significant change in TGE tissue, where net Na^+ flux was already zero. In the presence of theophylline, also added after glucose, the mean net Na^+ flux in control tissue decreased ($P < 0.005$); M→S Na^+ flux decreased ($P < 0.01$); S→M flux increased significantly ($P < 0.01$) compared with data on the same tissue in the presence of glucose. In TGE tissue after theophylline, M→S Na^+ flux decreased ($P < 0.001$), mean net flux changed from −0.2 to −2.2, which was barely significant ($P < 0.025$), and S→M flux did not change significantly. Net Na^+ flux did not differ significantly between the two study groups after theophylline.

Cl^- flux (table 1). Under basal conditions, unidirectional and net Cl^- fluxes in TGE tissue, like Na^+ fluxes, did not differ from those in control tissue. The controls responded to glucose with an increase in Cl^- M→S flux ($P < 0.01$), and decreased net Cl^- flux ($P < 0.025$), which remained secretory; TGE tissue failed to respond significantly to glucose. After ouabain the apparent inhibition of net Cl^- flux in control tissue and TGE was not significant although there was, in TGE tissue, a significant diminution in S→M flux ($P < 0.05$) compared with the previous glucose period. Theophylline provoked significant increases in S→M flux and net Cl^- secretion ($P < 0.001$) in both control and TGE tissue; mean increments in the two groups did not differ significantly.

TABLE 1. *Ion flux in short-circuited jejunal epithelium comparing tissue from TGE-infected pigs with controls*

Ion	Time (min)	Study conditions	No. animals	$J_{M \to S}$		$J_{S \to M}$		J_{net}	
				Mean	SE	Mean	SE	Mean	SE
				mEq cm⁻² hr⁻¹					
Sodium	0–45	Basal							
		Control	26	9.0	0.4	11.1	0.4	−2.1	0.3
		TGE	24	10.1	0.6	12.2	0.5	−2.1	0.4
		P[a]		NS		NS		NS	
	45–90	Glucose 30 mM							
		Control	26	13.0	0.4	10.3	0.4	+2.7	0.4
		TGE	24	10.2	0.6	10.4	0.5	−0.2	0.5
		P		<0.001		NS		<0.001	
	90–145	Ouabain 1 mM							
		Control	13	11.5	0.6	12.0	0.6	−0.5	0.6
		TGE	12	10.7	0.7	10.3	0.6	+0.3	0.6
		P		NS		<0.05		NS	
	90–145	Theophylline 10 mM							
		Control	13	11.2	0.5	12.2	0.8	−1.0	0.7
		TGE	12	7.0	0.7	9.2	0.9	−2.2	0.6
		P		<0.001		<0.025		NS	
Chloride	0–45	Basal							
		Control	18	5.7	0.4	9.5	0.3	−3.8	0.5
		TGE	16	5.0	0.6	8.6	0.6	−3.6	0.5
		P		NS		NS		NS	
	45–90	Glucose 30 mM							
		Control	18	7.1	0.3	9.3	0.3	−2.2	0.4
		TGE	16	4.6	0.5	7.8	0.5	−3.2	0.6
		P		<0.001		<0.025		NS	
	90–145	Ouabain 30 mM							
		Control	9	8.6	1.0	9.2	0.5	−0.6	1.0
		TGE	8	5.6	1.0	5.3	0.7	+0.3	0.7
		P		<0.05		<0.001		NS	
	90–145	Theophylline 10 mM							
		Control	9	8.2	0.9	14.9	1.0	−6.7	0.85
		TGE	8	5.2	1.0	11.2	1.3	−6.0	0.49
		P		<0.05		<0.05		NS	

[a] Compares control and infected tissue. NS, not significant.

Electrical data (fig. 1). Under basal conditions, PD's in the infected group were slightly but significantly greater than in controls ($P < 0.001$), while short-circuit currents (I_{sc}) did not differ between groups. After glucose the increments in both PD and I_{sc} were significantly less in tissue from infected pigs than in controls ($P < 0.001$). Theophylline provoked the same brisk increase in PD and I_{sc} in both groups; ouabain induced a sharp drop to zero in both. Conductance of infected tissue was lower than that of control tissue ($P < 0.005$) under all experimental conditions.

Na^+ Transport in enterocytes. Table 2 summarizes the Na^+ transport data from jejunal enterocytes isolated selectively from villi and studied in suspension. The Na^+ efflux rate constant under basal conditions was significantly less in the infected group compared with controls; in the presence of ouabain it decreased and became equal in the two groups. In the presence of glucose, 10 mM, the rate constant for control cells increased significantly over that found under basal conditions, while the rate constant for cells from infected pigs failed completely to respond to glucose.

Discussion

In jejunal epithelium, where previous in vivo experiments detected abnormal ion flux,[6] a defect in glucose-stimulated Na^+ transport has now been identified. In the Ussing chamber experiments, infected and control tissue secreted equally under basal conditions, the only significant difference between the two being a modest increase in PD across the infected tissue. However, when glucose was added, increments in net Na^+ and Cl^- fluxes, PD, and short circuit current in infected tissue were blunted. The data from enterocytes selectively harvested from villi of infected pigs, in which it was found that the response of Na^+ efflux to glucose was totally abolished, confirm the Ussing chamber findings and suggest that the defect extends along a sizable segment of gut and that it is particularly severe in cells from villi. Because the 30-cm segment from which the cells were taken was adjacent to but always below the segment used for short-circuit chamber experiments, it may be that the functional lesion is of greater severity in the more distal segment.

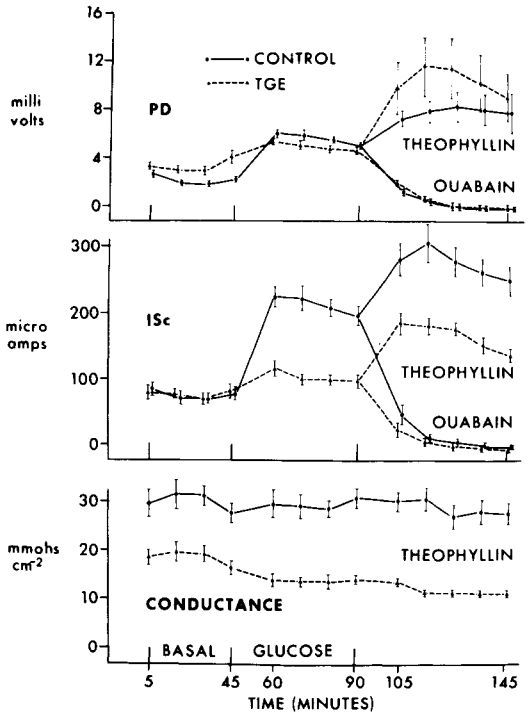

FIG. 1. Potential differences, short-circuit currents, and conductances in TGE-infected pigs compared with controls under basal conditions, in presence of glucose 20 mM, ouabain 1 mM, and theophylline 10 mM, plotted as means ± standard errors.

TABLE 2. *Na^+ efflux rate constants in suspensions of jejunal epithelial cells from control and infected animals ($^eK_{Na+}$ hr^{-1})*

	Na^+ efflux rate constant hr^{-1}		P^a
	Control	Infected	
	mean ± SE (no. animals)		
Mannitol 10 mM	11.0 ± 0.4 (22)	9.6 ± 0.5 (30)	<0.005
Glucose 10 mM	12.8 ± **0.5 (26)**[b]	9.5 ± 0.5 (27)	<0.001
Mannitol 10 mM plus ouabain 1 mM	4.9 ± 0.2 (51)	4.7 ± 0.3 (38)	NS

[a] Compares data from control and infected animals.
[b] Differs significantly from cells studied in mannitol 10 mM without ouabain ($P < 0.025$).

The in vitro findings could account for the decreased lumen-to-extracellular fluid Na^+ flux observed in intact pigs, since these previous in vivo experiments were done with glucose-containing perfusate. The failure to respond fully to glucose is undoubtedly a brush border defect, and there is additional evidence incriminating the brush border in TGE. When electron microscopic lesions occur in this disease, they are seen in the brush border and consist of shortened and sparse microvilli. Furthermore, several brush border enzymes are diminished in activity when measured in mucosal homgenates from diseased pigs.[5]

An additional factor contributing to decreased lumen-to-extracellular fluid Na^+ flux could be a defect in active Na^+ extrusion at the basal-lateral cell membrane. Previously, diminished Na-K-ATPase activity was found in mucosal homogenates from TGE-infected pigs.[5] In the present study, under basal conditions, ouabain-sensitive active Na^+ efflux was diminished in villous enterocytes from TGE pigs, suggesting a defective active Na^+ pump in TGE. Although decreased active Na^+ efflux could be explained by diminished intracellular Na^+ secondary to defective Na^+ influx, this explanation seems unlikely. Passive efflux, that portion of the efflux rate constant remaining after maximal inhibition with ouabain, is also dependent on intracellular Na^+ concentration, and it was not effected by TGE infection. It is believed that these latter data, consistent and reproducible, differ from those reported in an earlier report from this laboratory because previously the cells were exposed to an inappropriate temperature during the isolation period.[6]

The in vitro data do not fully explain the increased extracellular fluid-to-lumen Na^+ flux found in the previous marker perfusion study.[6] Presumably, the increased transmural PD occurring in TGE would encourage secretion under basal conditions. Either factors other than active epithelial transport are involved or possibly these methods were not sufficiently discriminating to detect significant abnormalities. Jejunum has rarely been studied in short-circuited chambers, and because diseased tissue was worked with it was necessary to eliminate basal control periods for each of the pharmacological agents used. Nevertheless, diseased tissue was stable over the lengthy study period and the control data agree generally with previous findings in the rat,[12] rabbit[13] and man.[14, 15] Of these only the rabbit jejunum has been found similar to the pig in that it secretes under basal conditions.

The transport defect in viral enteritis appears to differ from that occurring in response to the enterotoxins of *Vibrio cholerae*[15-18] or *Escherichia coli*.[19] In cholera, glucose-faciliated Na^+ transport is intact, and secretion is mediated by increased cellular concentrations of cyclic AMP; in ileal tissue exposed to choleragen, adenyl cyclase activity is increased and the normal electrical and transport responses to theophylline are suppressed. In TGE tissue, glucose-facilitated Na^+ transport is impaired, adenyl cyclase activity is normal,[6] and no convincing evidence of an abnormal response to theophylline has been found. The few available in vitro data on ion transport in other invasive enteric infections have not suggested common mechanisms for these diarrheas. In a granulomatous enteritis associated with Coccidia infestation in rabbit ileum, abnormal glucose-facilitated Na^+ transport and a normal electrical response to theophylline were found.[20] However, in a diarrheogenic invasive salmonella infection, glucose-faciliated Na^+ transport was normal and the response to theophylline was blunted.[21]

As the understanding of the response of the gut to viral invasion evolves, it will be possible to determine whether data from TGE diarrhea can be applied to the problem of the human infant with viral gastroenteritis. Recent

reports have clearly identified a specific virus as a major cause of acute infantile diarrhea throughout the world.[22-26] The disease in man, although caused by a different virus, bears many similarities to TGE in the pig; most notably it is an invasive infection of the proximal intestinal epithelium.

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