L-Glutamine Stimulates Jejunal Sodium and Chloride Absorption in Pig Rotavirus Enteritis

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Rotavirus enteritis is the leading cause of diarrhea in infants worldwide. A research priority of the World Health Organization is to develop oral rehydration solutions containing amino acids or other additives that will stimulate intestinal absorption more efficiently than the current glucose-based oral rehydration solutions. Glutamine is the principal metabolic fuel of the small bowel and a putative stimulator of mucosal repair. This report describes the transport response to mucosal L-glutamine following intestinal injury caused by porcine rotavirus. Peak symptoms and mucosal damage were observed 2–7 days after oral rotavirus inoculation. In vitro transport studies of the maximally injured region, the midjejunum (80% reduction in lactase), surprisingly, showed transport responses to L-glutamine (30 mmol/L) and L-alanine (30 mmol/L) that were similar qualitatively and quantitatively to those observed in control tissue. Subsequent application of mucosal D-glucose (30 mmol/L) caused additional stimulation of electrogenic Na$^+$ transport, but the response to glucose was blunted ($P < 0.05$) in the infected tissues. Glutamine and alanine enhanced Na$^+$ absorption to a similar degree (2–2.5 μEq $\cdot$ cm^{-2} $\cdot$ h^{-1}), but glutamine stimulated equal amounts of electrogenic and electroneutral NaCl absorption, whereas alanine had no significant effect on net Cl$^-$ flux. Glutamine is a potentially useful substrate for investigation in oral rehydration solutions for infant diarrhea.

Viral enteritis is the leading cause of diarrhea in infants and toddlers less than 2 years old (1). Each year in the United States, about 22,000 infants are hospitalized for treatment of rotavirus-induced dehydration (2). Central to the pathophysiology of diarrhea in one experimental model of viral enteritis, transmissible gastroenteritis (TGE), are the following intestinal abnormalities: impaired glucose and amino acid–coupled Na$^+$ transport (3–4), defective NaCl absorption (5), diminished disaccharide hydrolysis (6–8), and reduced mucosal absorptive surface (4,8). These abnormalities differ from those in enterotoxigenic diarrheas, in which Cl$^-$ is actively secreted but mucosal surface area and Na$^+$-coupled organic solute transport are preserved.

Glutamine, the chief metabolic fuel of the small intestine (9), is an amino acid which cotransports Na$^+$ across the enterocyte brush border membrane (10). We compared the transport response to L-glutamine with the transport response to L-alanine. Compelling reasons to study glutamine in viral diarrhea include the observations that glutamine has been reported to stimulate intestinal mucosal growth and repair after chemotherapy-induced villus damage in rats (11) and that glutamine is a potent stimulator of electroneutral NaCl absorption in the normal newborn pig jejunum (12). In the current study, we characterized the intestinal response to L-glutamine in piglet rotavirus enteritis, a naturally occurring disease of major importance in newborn swine.

Materials and Methods

Animals

Experiments were designed to prevent sow-acquired rotavirus infection. Piglets were reared in individual feed-

ing cages and fed artificial formula (13). Cross-bred sows in a barrier-intact intensive care farrowing facility were washed with povidone-iodine before delivery. The newborn piglets were caught on towels, taken to an isolation facility, caged, and fed [by an automatic feeding device (Autosow; Nutritech, Raleigh, NC)] 12.5 mL $\cdot$ kg^{-1} $\cdot$ h^{-1} of an artificial formula, patterned after sow milk, which contained 20% solids composed of 30% protein, 40% lactose, 20% fat, electrolytes, trace minerals, and vitamins (13).

On day 4 after birth, experimental animals were administered approximately 10^6 rotavirus particles in 10-mL diet. The aliquots were obtained from a frozen stock of bacteria-free fecal supernatant from rotavirus-infected piglets. Previous studies showed that animals infected in the first week of life uniformly experience viral enteritis (14), although pigs infected later in life may also become ill. Body weight was checked every other day, and stool consistency was graded as follows: 1, solid feces; 2, soft, looser than normal stools; and 3, liquid diarrheal feces. Stool was assayed for rotavirus antigen by Virogen Rotatest (Wampole Laboratories, Cranbury, NJ). Rotavirus positivity was scored 0+ to 4+ (4+ signifying a strongly positive result), based on the speed of agglutination.

Animals were fasted for 6–8 hours before the intestine was removed for enzyme and transport studies. They were then given 50–70 mg ketamine IM and 2–4 minutes later were administered a lethal injection of 2–3 g sodium pentobarbital. Following an abdominal incision, the entire small bowel beginning 10 cm distal to the suspensory ligament was removed and flushed with ice-cold normal saline. The first 10 cm of proximal jejunum, a 20-cm segment obtained at the midpoint of the small intestine, and the distal 10 cm of terminal ileum were removed and placed in oxygenated Ringer's buffer; three 10-cm segments were scraped and homogenized for enzyme assays, as described below. The remaining 10 cm of the midjejunum was placed in oxygenated transport buffer for ion flux studies. A 0.5–1-cm segment was placed in 10% formalin for light microscopic evaluation.

Enzyme Assays

Mucosa was scraped from the jejunal segments with a glass slide, divided in half, and homogenized in two buffers (0.1 g mucosa/mL buffer). The first sample was homogenized in 0.25 mol/L sucrose, 0.2 mol/L Tris, 0.15 mol/L KCl, 2.5 mmol/L ethylenediaminetetraacetic acid (EDTA), pH 7.4, at 4°C; immediately frozen in aliquots at −70°C; and assayed within 2–3 weeks for sucrase (15), lactase (15), and total and ouabain-inhibitable adenosine triphosphatase (Na$^+$,K$^+$-ATPase) activities (8). A second mucosal scraping was homogenized and frozen in 0.25 mol/L sucrose, 0.2 mol/L Tris, 0.15 mol/L KCl, pH 7.4, and was later used to determine thymidine kinase activity (16). Protein was measured by the method of Lowry et al. (17).

Electrical Measurements

Jejunum for transport studies was drawn over a plastic rod, incised longitudinally, and stripped of muscu-laris propria. Segments mounted between lucite half-chambers exposing an area of 1.13 cm^2 were bathed at 37°C in 10 mL of oxygenated buffer containing (in mmol/L) Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO$_3$, 25; H$_2$PO$_4$, 0.4; and HPO$_4$, 2.4, pH 7.4. To provide metabolic fuel, 10 mmol/L glucose was added to the serosal buffer before the start of each experiment, with equimolar mannitol added on the mucosal side. Potential difference (PD) across the tissue was measured by calomel electrodes in saturated KCl, attached to agar bridges (4 g/dL, made up in Ringer's buffer) positioned near the surface of the tissue. The tissue was continuously short-circuited with automatic voltage clamps (World Precision Instruments, New Haven, CT), which compensated for the fluid resistance. Clamps introduced current to half-cells through Ag-AgCl electrodes which were connected to the chambers via agar bridges. All tissue sheets were mounted in the chambers within 30 minutes of the piglet's death and were continuously short-circuited, except for 5-second intervals every 20 minutes, when the open circuit PD was measured. Conductance (G) was calculated from the open circuit PD and short-circuit current (Isc) using Ohm's law. Transmucosal PD and Isc were stable for the entire study period, in both control and infected preparations.

Transport Studies

Chambers were paired so that tissue conductance did not differ by more than 25%, and data were discarded if conductance in a chamber pair differed by more than 25% during the experiment. Fifteen to twenty minutes after mounting the tissue, ^{22}NaCl (2 μCi) and Na^{36}Cl (2 μCi) were added to either the mucosal or serosal reservoir. After 15–20 minutes of equilibration, unidirectional Na$^+$ and Cl$^-$ fluxes were calculated from isotopic determinations of 1.0-mL samples drawn at 20-minute intervals.

At 40 minutes, 30 mmol/L L-glutamine or L-alanine powder was added to the mucosal side, balanced by equimolar mannitol on the opposite side. This concentration of amino acid was chosen because previous studies showed maximal electrical and Na$^+$ transport response to L-alanine (4), L-glutamine (12), and D-glucose (4,12) at 20–30 mmol/L in the proximal piglet jejunum. Twenty minutes later, we measured unidirectional ion fluxes at 20-minute intervals for a subsequent 40-minute period. Fluxes from mucosa to serosa (J$_{ms}$) and from serosa to mucosa (J$_{sm}$) were determined from paired chambers, and J$_{net}$ was calculated. At 100 minutes, D-glucose (30 mmol/L) was added to both the mucosal and serosal baths, and Isc was determined for a subsequent 20-minute period.

Histological Studies

Jejunal strips were fixed in 10% formalin, blocked in paraffin, and stained with H&E. A single observer (E.K.), blinded to the age of the animals, measured 6–10 representative, properly oriented crypts and villi using light microscopy with a Videoplan computer-assisted morphometry system (Carl Zeiss, Oberkochen, Germany).

Statistics

Wilcoxon signed rank test was used on matched flux data pairs to assess the significance of the mean flux increments. This test was chosen because it is a nonparametric test and therefore does not assume a normal distribution. Short-circuit current data for glucose response in control and infected pigs were compared using Mann–Whitney U test for unpaired data. Data are summarized as mean $\pm$ SEM. Results of the tests are expressed as P values.

Chemicals

All unlabeled chemicals were obtained from Sigma, St. Louis, MO. $^{22}NaCl$ and $Na^{36}Cl$ were obtained from Amersham, Arlington Heights, IL, and ICN Radiochemicals, Irvine, CA, respectively.

These studies were approved by the University of North Carolina Animal Care Committee.

Results

Clinical Manifestations of Rotavirus Diarrhea

Thirteen piglets were inoculated with rotavirus; the infection rate was 100% (Figure 1). None of the animals died. Five piglets vomited once 2–3 days after inoculation, just before the onset of the diarrhea. Watery diarrhea ensued 48 hours after inoculation in most cases, typically lasting 3–6 days. In all but one animal, rotavirus shedding began 4–8 hours after
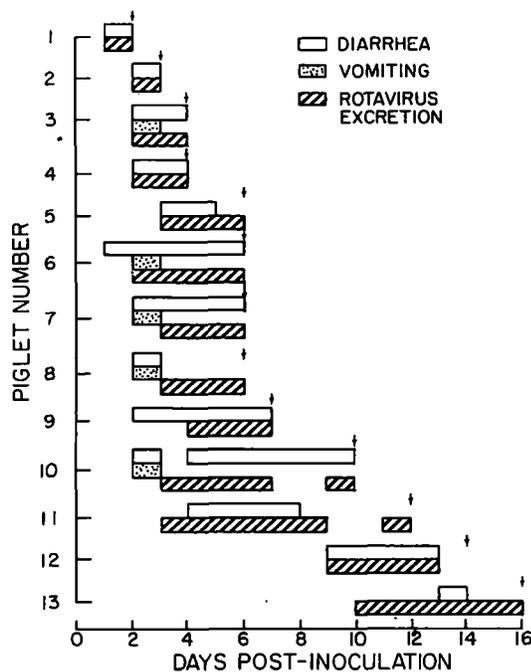
Figure 1. Clinical manifestations of porcine rotavirus enteritis. Pigs were orally inoculated with virus on day 4 of life and killed on the days indicated by *arrows*. Presence of symptoms on a particular day is indicated by *bar graph*.

Table 1. Effect of Experimental Piglet Rotavirus Infection on Midjejunal Crypt-Villus Dimensions

Group	Villus height (μm)	Crypt depth (μm)
Control (20)	441 $\pm$ 157	172 $\pm$ 41
Rotavirus (8)	154 $\pm$ 38	173 $\pm$ 21
P	<0.01	NS

diarrhea commenced and typically lasted 4–8 days. Four days after inoculation, infected pigs had gained 420 $\pm$ 90 g in body wt, whereas uninfected controls had gained 500 $\pm$ 30 g ($P = $ NS).

Fecal excretion of rotavirus was present at the time of death in all but one animal. High titer excretion (3+–4+ scores) occurred in 12 of 13 pigs. Fecal excretion of rotavirus, in two cases, resolved and later recurred. Only one animal was not excreting virus at the time of death.

Jejunal Mucosal Villus-Crypt Dimensions

Microscopic evaluation of histological preparations from eight pigs (7–16 days of age), infected with rotavirus on day 4, and from 20 age-matched uninfected controls (6–15 days of age) demonstrated that rotavirus inoculation resulted in significant blunting of the midjejunal villi (by 50%) and no significant change in crypt depth (Table 1). Figure 2 shows the histological appearance of the jejunum from a normal pig and a piglet infected 4 days earlier. Whereas the normal midjejunal villus-crypt ratio was approximately 3:1, viral inoculation resulted in severe mucosal thinning, with a villus-crypt ratio of about 1:1. In addition, focal ballooning degeneration of enterocytes was noted, predominantly in areas between plicae. On the plicae, degeneration was less evident. Viral cytopathic effect was most prominent in the villus tips.

Intestinal Enzymes

Rotavirus infection significantly altered mucosal enzyme profiles in the proximal and midjejunum and in the ileum. Table 2 shows enzyme data from the pigs described in Figure 1 and several other piglets infected on day 4 and killed <16 days after inoculation. In proximal jejunum, we observed a significant reduction in Na^+,K^+-ATPase activity, whereas alkaline phosphatase activity was unaltered. Although lactase specific activity appeared reduced, the difference between control and infected animals was not statistically significant. In the ileum, rotavirus-infected tissues demonstrated significant reductions in both alkaline phosphatase and Na^+,K^+-ATPase activities; thymidine kinase, a crypt cell enzyme involved in mucosal regeneration (5,18), was elevated
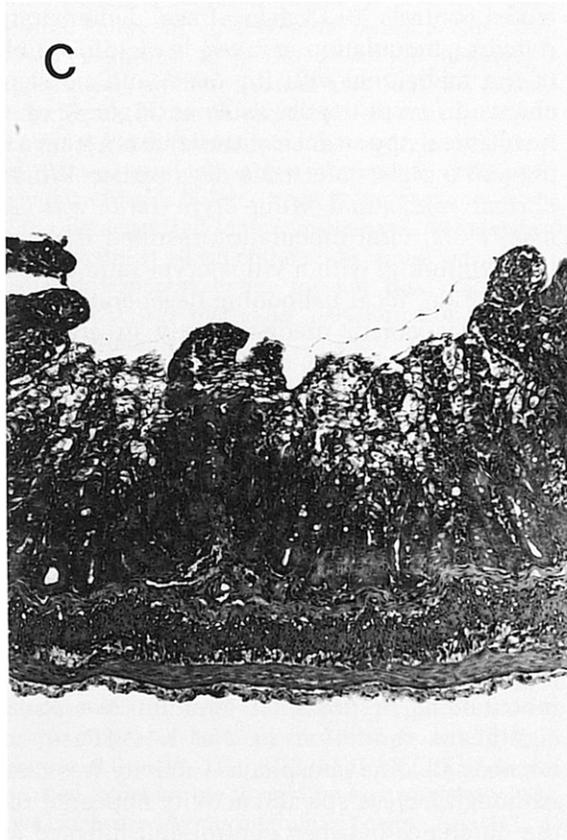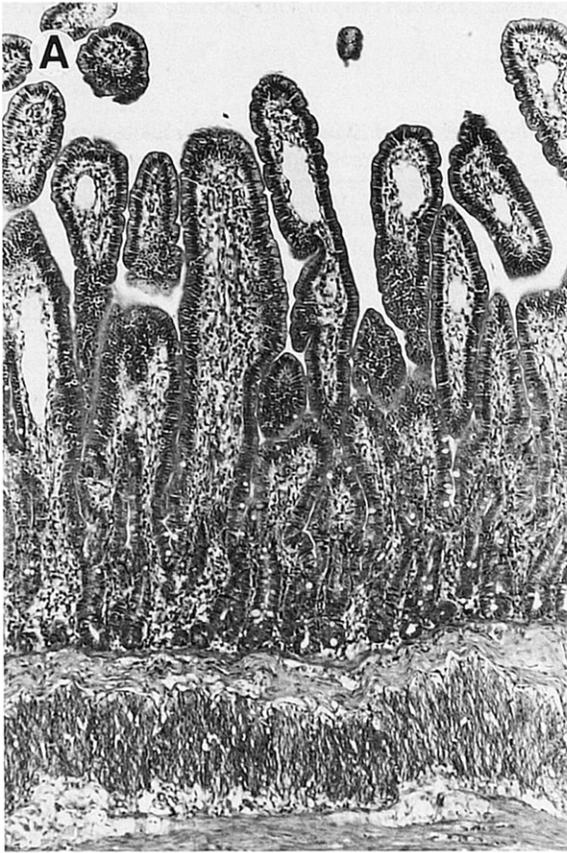
Figure 2. *A*. Midjejunal mucosa of normal 2-week-old piglet. There is normal thickness of the mucosa and a normal villus-crypt ratio (H&E; original magnification ×100).
B. The villus architecture and enterocyte morphology are unremarkable (H&E; original magnification ×400).
C. Porcine rotavirus enteritis in a 9-day-old piglet (5 days after inoculation). There is marked thinning of the mucosa with focal ballooning degeneration of enterocytes. Villus height is decreased without significant change in the crypt height (H&E; original magnification ×100).
D. Viral cytopathic effect is most prominent in the villus tips (H&E; original magnification ×400).

Table 2. Comparison of Specific Activities of Mucosal Enzymes in Porcine Rotavirus-Infected and Control Piglets

Intestinal segment	Lactase ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Alkaline phosphatase ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	Na^+,K^+-ATPase ($nmol \cdot mg^{-1} \cdot min^{-1}$)	Thymidine kinase ($pmol \cdot mg^{-1} \cdot min^{-1}$)
Proximal				
Control	110 ± 11 (7)	19 ± 2 (7)	175 ± 13 (7)	5 ± 1 (7)
Rotavirus	61 ± 27 (4)	19 ± 3 (4)	94 ± 14 (4)	8 ± 3 (4)
P	NS	NS	<0.005	NS
Mid				
Control	203 ± 26 (10)	36 ± 2 (10)	73 ± 8 (5)	2 ± 0.1 (12)
Rotavirus	39 ± 13 (9)	14 ± 2 (18)	39 ± 6 (16)	15 ± 5 (21)
P	<0.001	<0.001	<0.01	<0.001
Distal				
Control	42 ± 13 (5)	30 ± 5 (7)	68 ± 5 (7)	4 ± 1 (7)
Rotavirus	10 ± 2 (4)	16 ± 3 (11)	45 ± 6 (11)	13 ± 2 (11)
P	NS	<0.005	<0.05	<0.01

NOTE. Values are means ± SE. All piglets in the rotavirus group were infected on day 4 of life and killed 2–16 days after inoculation (i.e., on days 6–20 of age). Control pigs are 6–15 days old. Number of animals studied is in parentheses. For further details, see Materials and Methods.

threefold. Even in pigs studied as late as 15 days after inoculation, mucosal enzymes remained markedly abnormal.

The most severely damaged tissue was the midjejunum. Here, homogenate lactase specific activity was reduced to about 20% of normal for age, and alkaline phosphatase and Na^+,K^+-ATPase activities were also reduced significantly. Thymidine kinase activity was increased sevenfold.

Analysis of the effect of time after inoculation on enzyme activities showed that all pigs killed before 9 days after inoculation had similar levels of brush border enzymes and Na^+,K^+-ATPase; however, one of the two pigs developing diarrhea >9 days after inoculation had a milder degree of damage as assessed by mucosal enzymes. Thymidine kinase, in all parts of the bowel, peaked at 2–7 days after inoculation but remained elevated even in animals killed as late as 16 days after inoculation. Figure 3 shows thymidine kinase activities from homogenates of the midjejunum. Subsequent data (n = 9 pigs) showed that lactase activity remained <50% of normal in pigs studied 2–3 weeks after inoculation.

Transport Studies

Control (7–12-day-old) piglet midjejunum, like the proximal jejunum in previous studies (5), secreted Cl^- and, to a lesser extent, secreted Na^+ under basal conditions (Table 3). Tissue conductance after L-glutamine increased by 40%–50%.

L-Glutamine augmented net Na^+ and Cl^- transport ($P < 0.005$ and $P < 0.025$, respectively) via increased mucosal-to-serosal Na^+ and Cl^- fluxes. There was an increase in J_{sm}^{Na}, which could represent increased flux across the paracellular pathway and/or stimulation of

a transcellular Na^+ secretory process. The magnitude of the increase in J_{sm}^{Na} was less than the increase in J_{ms}^{Na}. Analysis of the Na^+ absorptive response to glutamine showed that J_{net}^{Na} after glutamine increased more than Isc (2.6 ± 0.5 compared with 2.0 ± 0.5 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$). While this difference was not significant, it should be noted that glutamine stimulated a significant increase in net Cl^- flux (1.0 ± 0.3 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$). This transmucosal absorption of Cl^- in parallel with Na^+ is an electrically silent (electroneutral) NaCl-absorptive response to glutamine. The en-
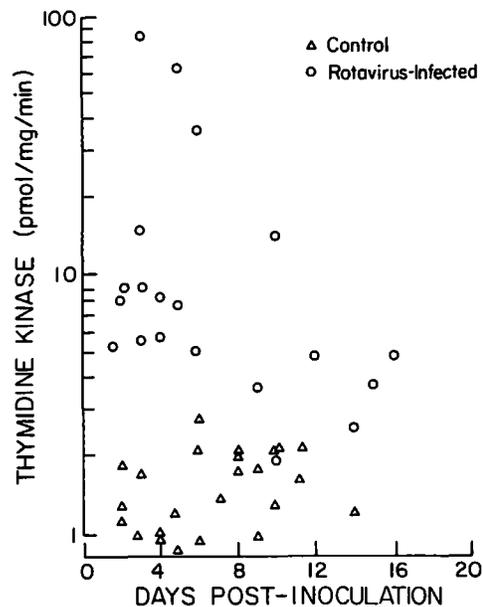

Figure 3. Mucosal thymidine kinase specific activities in midjejunum (*circles*), plotted against day after rotavirus inoculation. Comparison values in age-matched uninfected tissue are shown in *triangles*.

Table 3. Control Piglet Jejunum: Transport Response to Mucosal L-Glutamine (30 mmol/L) and L-Alanine (30 mmol/L)

	n	J_{ms}^{Na}	J_{sm}^{Na}	J_{net}^{Na}	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}	J_{net}^{R}	Isc	G
Basal		6.2 ± 0.5	8.2 ± 0.6	−1.9 ± 0.6	4.1 ± 0.3	8.0 ± 0.7	−3.9 ± 0.7	0.7 ± 0.4	2.7 ± 0.3	17.6 ± 1.3
L-Glutamine	8	10.4 ± 1.2	9.8 ± 0.8	0.7 ± 0.8	5.9 ± 0.5	8.8 ± 0.8	−2.9 ± 0.7	1.1 ± 0.8	4.7 ± 0.6	26.0 ± 2.2
Δ		4.2 ± 1.0	1.6 ± 0.6	2.6 ± 0.5	1.8 ± 0.3	0.8 ± 0.5	1.0 ± 0.3	0.4 ± 0.7	2.0 ± 0.5	8.4 ± 1.6
P		<0.005	<0.025	<0.005	<0.005	NS	<0.025	NS	<0.005	<0.005
Basal		6.6 ± 0.2	8.6 ± 0.8	−2.0 ± 0.7	5.0 ± 0.3	7.7 ± 1.3	−2.7 ± 1.5	1.4 ± 1.3	2.1 ± 0.4	18.7 ± 0.7
L-Alanine	4	9.5 ± 0.8	9.7 ± 0.9	−0.1 ± 0.7	5.3 ± 0.3	8.7 ± 1.4	−3.5 ± 1.5	1.8 ± 1.6	5.1 ± 0.8	22.5 ± 2.0
Δ		3.0 ± 0.6	1.1 ± 0.4	1.8 ± 0.6	0.3 ± 0.3	1.1 ± 0.3	−0.8 ± 0.4	0.3 ± 0.6	3.0 ± 0.5	3.8 ± 1.3
P		<0.05	<0.05	<0.05	NS	<0.05	NS	NS	<0.05	<0.05

NOTE. Values are means + SE. Units for ion fluxes and Isc are $\mu Eq \cdot cm^{-2} \cdot h^{-1}$; units for G are mS/cm^2. Wilcoxon signed rank test was used to determine the statistical significance of the difference caused by L-glutamine or L-alanine. Pigs are 7–12 days old.

hancement of neutral NaCl absorption after glutamine was previously found to be even greater in the proximal piglet jejunum (ca 2 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$) (12).

L-alanine, in contrast to L-glutamine, stimulated electrogenic Na^+ flux, via an increase in mucosal-to-serosal Na^+ flux, but did not alter mucosal-to-serosal or net Cl^- fluxes. Like glutamine, alanine increased tissue conductance and serosal-to-mucosal fluxes of Na^+. Alanine did not alter J_{ms}^{Cl} significantly, and although J_{net}^{Cl} consistently decreased after this amino acid, the decrease in net Cl^- flux after alanine did not reach statistical significance. However, alanine significantly enhanced Cl^- flux in the secretory direction ($\Delta J_{sm}^{Cl} = 1.1 \ \mu Eq \cdot cm^{-2} \cdot h^{-1}$; $P < 0.05$) in normal piglet jejunum.

Jejunal response in tissues of age-matched rotavirus-infected animals was studied 2–7 days after inoculation (Table 4). Basal electrical parameters and Na^+ and Cl^- fluxes were virtually identical to those in control midjejuna. To our surprise, we found that responses to the two amino acids were qualitatively and quantitatively quite similar to those in uninfected tissue. L-glutamine increased tissue conductance, whereas L-alanine did not significantly alter conductance. In addition, the infected tissue demonstrated an increase in serosal-to-mucosal Na^+ flux (ca 2 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$).

L-alanine, in rotavirus-damaged tissues, stimulated net Na^+ flux (2.7 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$) while not signifi-

cantly altering net Cl^- flux. In contrast, L-glutamine augmented both Na^+ (2.3 $\mu Eq \cdot cm^{-2} \cdot h$) and Cl^- flux (1.2 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$) significantly. The finding of increased Isc and increased Na^+ and Cl^- transport are compatible with dual transport effects of glutamine, whereby electrogenic (glutamine-Na^+) cotransport and also electroneutral NaCl absorption are stimulated by the amino acid.

In the small intestine, mucosal glucose stimulates electrogenic Na^+ absorption. The increase in J_{net}^{Na} after glucose equals the increase in Isc (19). In our studies (Figure 4), when D-glucose (30 mmol/L) was added after L-glutamine, we found that both control and infected tissues responded to glucose with an increase in Isc. However, response in controls exceeded response in the rotavirus-infected group by more than twofold ($P < 0.05$).

Discussion

Colostrum-deprived piglets provide a particularly useful model of enteritis, because each animal is agammaglobulinemic and therefore susceptible to viral invasion and mucosal injury. Our studies of swine rotavirus enteritis in newborn artificially reared piglets are consistent with the findings in other piglet enteridites (coronavirus and TGE enteritis, human rotavirus enteritis in conventional pigs (20), and porcine rotavirus enteritis in gnotobiotic piglets (21)).

Table 4. Rotavirus-Infected Piglet Jejunal Transport Response to Mucosal L-Glutamine (30 mmol/L) and L-Alanine (30 mmol/L)

	n	J_{ms}^{Na}	J_{sm}^{Na}	J_{net}^{Na}	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}	J_{net}^{R}	Isc	G
Basal		8.5 ± 1.9	9.6 ± 1.1	−1.1 ± 0.5	5.0 ± 0.6	7.3 ± 1.0	−2.3 ± 0.9	0.6 ± 0.5	1.9 ± 0.3	19.4 ± 2.1
L-Glutamine	6	13.1 ± 1.5	11.8 ± 1.2	1.3 ± 0.7	6.8 ± 0.5	7.9 ± 1.0	−1.2 ± 0.9	1.4 ± 0.7	3.8 ± 0.7	27.8 ± 2.7
Δ		4.6 ± 0.8	2.3 ± 0.4	2.3 ± 0.4	1.8± 0.2	0.6 ± 0.3	1.2 ± 0.4	0.8 ± 0.5	1.9 ± 0.5	8.4 ± 1.7
P		<0.005	<0.01	<0.005	<0.001	NS	<0.025	NS	<0.01	0.005
Basal		7.4 ± 0.9	9.2 ± 0.8	−1.9 ± 0.5	4.6 ± 0.5	6.7 ± 0.7	−2.1 ± 1.0	1.7 ± 0.6	1.9 ± 0.3	18.2 ± 1.6
L-Alanine	8	11.6 ± 1.2	10.8 ± 0.7	0.8 ± 0.8	5.5 ± 0.4	8.0 ± 0.8	−2.6 ± 1.0	1.8 ± 0.7	5.2 ± 0.9	23.1 ± 2.6
Δ		4.3 ± 0.7	1.5 ± 0.4	2.7 ± 0.9	0.8 ± 0.2	1.3 ± 0.3	−0.5 ± 0.3	0.1 ± 0.6	3.3 ± 0.7	4.8 ± 2.1
P		<0.001	<0.01	<0.025	<0.01	<0.005	NS	NS	<0.005	NS

NOTE. Values are means ± SE. See legend to Table 3 for additional information. Pigs are 6–11 days old, each infected at 4 days of age.

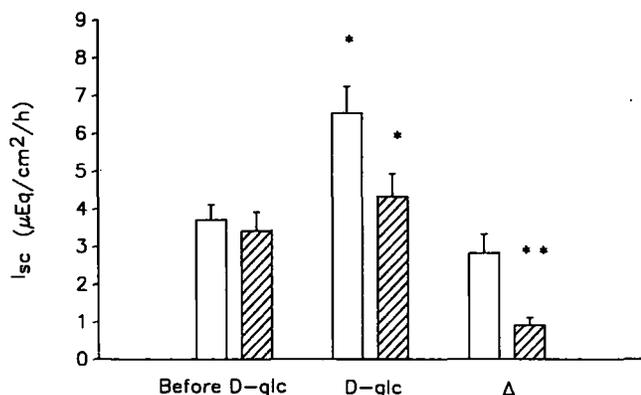Figure 4. Piglet jejunal short-circuit current (Isc) response to mucosal D-glucose (30 mmol/L). "Before D-glucose" refers to time point 100 minutes, when tissue bath contains normal Ringer's solution and 30 mmol/L L-glutamine. D-glucose (30 mmol/L) is added to mucosal and serosal baths, and the resulting Isc 15 minutes later is shown (mean ± SE). $*P < 0.05$ compared with Isc before D-glucose; $**P < 0.05$ when responses of normal and infected tissues are compared. □, Control (n = 13); ▨, rotavirus-infected (n = 9).

In piglet models of diarrhea, the epithelium is dominated by crypt-type enterocytes, with villus blunting, increased specific activity of an enzyme involved in DNA replication (thymidine kinase), and significantly reduced brush border hydrolases and basolateral Na^+,K^+-ATPase activities. Infant mice infected with rotavirus appear to develop a much milder lesion (22,23). Biopsy data obtained from human infants suffering from rotavirus enteritis show abnormalities similar to the porcine lesion, although a spectrum of villus damage is seen (24).

Previous studies of transport in proximal jejuna of normal pigs showed that stripped jejunum in Ussing chambers secretes Cl^- and, to a lesser extent, Na^+ (5,12,25). Na^+ secretion persists in the absence of Cl^-, is associated with a residual flux, and is abolished in the nominal absence of HCO_3, suggesting that pig jejunum secretes $NaHCO_3$ in an electrogenic manner (25). $Na-(HCO_3^-)_3$ secretion is well established in the renal tubule (26). As in our previous studies of piglet TGE (4,5), we found no differences in basal ion transport, comparing rotavirus-infected with normal jejunum.

Reduced intestinal glucose-stimulated Na^+ transport has been shown to be the most consistent functional abnormality in previous studies of TGE enteritis (3,18). Brush border membrane vesicle studies indicated the loss of a high-affinity glucose-Na^+ cotransporter (7). In the current studies, we determined that the Isc response to mucosal glucose is abnormal (Figure 4) (in the presence of glutamine) in the rotavirus-infected gut. The observation is compatible with a defect in epithelial glucose-Na^+ cotransport and/or to a reduced maximal transport capacity

in the rotavirus-infected gut. Potential contributing factors include a brush border carrier defect and the observed 40% reduction of Na^+,K "pump" activity in diseased tissue.

The major goal of our study was to compare the effects on transport of the two amino acids L-alanine and L-glutamine. Our data confirm that amino acid and glucose have combined effects in enhancing Na^+ absorption across the virus-damaged gut. Glutamine and alanine were equally effective in vitro stimulators of Na^+ absorption in control and rotavirus-infected jejuna. Glutamine stimulates Na^+ transport in newborn piglet proximal jejunum primarily by an electroneutral, rather than electrogenic Na^+-coupled mechanism (12); serosal glutamine, we have found, will also increase NaCl transport (12). In the mid intestine, the current studies demonstrate that electrogenic glutamine-Na^+ cotransport predominates over NaCl absorption. Although we have consistently observed a NaCl absorptive response to glutamine, whether it is added to the mucosal or serosal sides of the tissue, we have shown that Cl^- absorption does not increase after mucosal phenylalanine or glucose. The latter two substrates, in pig jejunum, are transported solely by a Na^+-coupled mechanism (12).

We suggest that either transmucosal NaCl absorption or coupled Na^+/H^+, Cl^-/HCO_3^- exchanges may be stimulated by glutamine in rotavirus-injured jejunum. MacLeod and Hamilton found in coronavirus-damaged piglet small bowel that mucosal-to-serosal Na^+ and Cl^- fluxes were low and could not be reduced further by furosemide or theophylline (5). They speculated that jejunal epithelial cell NaCl absorption may be absent in severe viral enteritis. One possible explanation for this apparent discrepancy between our findings and theirs is that brush border NaCl transport in TGE may be present but inhibited in the basal state. Transmucosal NaCl absorption could be inhibited by factors released in the subepithelium, such as immune cell mediators or neurotransmitters discharged from enteric nerves (27). Mucosal glutamine may counteract this inhibition, by altering the metabolism of epithelial or lamina propria cells.

The brush border membrane of differentiated villus cells is the primary site of glucose-facilitated Na^+ absorption (7). If amino acid-Na^+ cotransport is primarily carried out at the villus tip, as suggested by the autoradiographic studies of Smith (28), one might have anticipated that the rotavirus-infected preparations would have responded poorly to glutamine and alanine. However, we observed a normal transport response in the infected jejunum (Table 4). A normal increment in absorption in an epithelium with reduced villus surface area strongly suggests the enterocytes populating the damaged tissue are actually

responding with increased glutamine-stimulated Na^+ transport.

Our data do not explain this robust transport response; however, a potentially relevant finding is that the specific activity of phosphate-dependent glutaminase, the first and rate-limiting enzyme for mitochondrial glutamine oxidation, is maximal in cells isolated from the villus-crypt junction (29). Nagy et al. found that oxidation of labeled glutamine to CO_2 was increased 75% in cells at the crypt neck compared with villus or crypt cells (29). The rapid intracellular metabolism of this important fuel by crypt-type cells could, in theory, stimulate the Na^+,K^+-ATPase and increase electrolyte absorption or could enhance L-glutamine-Na^+ entry by preventing intracellular accumulation of the amino acid.

Oral rehydration therapy (ORT), one of the major medical advances of the century, is believed to promote gut absorption because of the high concentrations of both glucose and Na^+, substrates which are transported in a coupled manner across the brush border (19). Unfortunately, when the bowel is injured by common pathogens such as rotavirus, infants administered standard ORT malabsorb at least 10% of ingested glucose (30). Recent attempts at improving ORT have been based on the assumption that adding one or more amino acids cotransported with Na^+ to standard ORT could promote greater absorptive flows of Na^+ and water (31,32). For example, a solution containing glucose and L-alanine, in a large double-blind trial, was shown to be more effective than standard glucose ORT in human cholera (32).

The current data show no impairment of the alanine response in rotavirus enteritis. We previously found blunted alanine-stimulated Na^+ transport in acute TGE enteritis in pigs (4); however, our enzyme data suggest that rotavirus enteritis induces a milder lesion in pigs than TGE. Of possible concern regarding the use of L-alanine in the treatment of viral enteritis in infants is the observation that L-alanine consistently increases J_{sm}^{Cl} (Tables 3 and 4). Although a statistically significant effect of alanine on net Cl^- secretion across the mucosal barrier was not demonstrated in the current studies, L-alanine is a recognized activator of Cl^- channels in enterocytes (33). Furthermore, in the ileum of newborn rabbits, a tissue with a much higher transmural conductance than piglet jejunum, alanine has no effect on Na^+ absorption and significantly increases transepithelial Cl^- secretion (34). This finding is of concern if an oral rehydration solution (ORS) containing alanine and glucose are to be studied in infants.

On the basis of these in vitro findings, we speculate that an ORS containing glutamine and glucose might be superior to conventional· glucose ORT in viral enteritis. Glutamine appears to be an effective stimula-

tor of Na^+ and NaCl transport in rotavirus-damaged jejunum.

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