Intestinal glucose transport in acute viral enteritis in piglets

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Summary
1. We studied intestinal glucose transport in pigs during the acute and convalescent phases of an invasive viral enteritis, transmissible gastro-enteritis.

2. When diarrhoea was severe 40 h after experimental infection, net absorption of glucose, Na⁺ and water, measured by marker perfusion in the jejunum, was reduced; the enhancement of Na⁺ and water absorption in response to increasing perfusate glucose concentrations up to 120 mmol/l was diminished compared with the response observed in control and convalescent pigs.

3. Measured in vitro, 40 h after infection, unidirectional fluxes of 3-O-methyl-D-glucose across the jejunal epithelium were reduced and net absorption of the sugar was obliterated. Phlorizin (0.05 mmol/l), which completely inhibited net 3-O-methyl-D-glucose absorption in control tissue, had no significant effect on transmissible gastroenteritis jejunum.

4. Our data suggest that in this invasive viral enteritis, which closely resembles human rotavirus enteritis, glucose absorption is impaired as a result of defects in both active and passive glucose flux.

5. Differences between the mechanisms of viral diarrhoea, demonstrated by our study and those of the enterotoxigenic diarrhoeas, should be taken into consideration in formulating active therapeutic measures for children with acute viral diarrhoea.

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Key words: glucose transport, jejunum, transmissible gastroenteritis, viral enteritis.

Introduction
Transmissible gastroenteritis virus, a corona virus, invades the small-intestinal epithelium of young pigs causing watery diarrhoea [1, 2]. Medical research interest in this veterinary disease has been stimulated by the striking clinical and pathological similarities noted between transmissible gastroenteritis and human rotavirus diarrhoea [3, 4]. Certain epithelial events that determine these acute viral diarrhoeas have already been documented in experimentally infected piglets. In both transmissible gastroenteritis and human rotavirus enteritis the major defects of intestinal function develop after the virus has been shed into the intestinal lumen; they coincide with the appearance on villi of immature crypt-type enterocytes [3, 5]. Diminished mucosal (Na⁺, K⁺-activated)-ATPase activity and defective glucose-stimulated Na⁺ transport are observed consistently in acute transmissible gastroenteritis whether or not a structural mucosal lesion is seen [1, 6]. An abnormality of glucose transport would also be expected in transmissible gastroenteritis, since the transport of glucose and Na⁺ at the brush border are dependent on one another [7]. In the present study we measured jejunal glucose transport in vivo and in vitro during the course of experimentally induced transmissible gastroenteritis in young pigs. Our goal was to evaluate the role of glucose transport in the pathogenesis of acute viral diarrhoea.
Materials and methods

Experiments in vivo

At 7–8 days of age, eight conventional York piglets from three litters were weaned on to reconstituted whole evaporated cow's milk formula and polyvinyl triple-lumen tubes (with 10 cm mixing segments and 20 cm test segments) were securely fixed in the upper jejunum, 30 cm beyond the ligament of Treitz [2]. Studies were begun on day 6 postoperatively when the piglets had fully recovered from surgery. The animals were fasted for 10 h before each study. Each animal was studied before infection (control phase), when diarrhoea was severe (acute phase) 49 h after receiving transmissible gastroenteritis virus via a stomach tube and three were also studied 144 h after infection when acute symptoms had subsided (convalescent phase).

Net intestinal glucose, water and Na⁺ fluxes were measured by a marker perfusion technique [2]. Infused at 1–8 ml/min, the perfused solutions contained Na⁺ (115 mmol/l), chloride (110 mmol/l), bicarbonate (10 mmol/l), potassium (5 mmol/l), 3 µCi of [³H]polyethylene glycol 4000/l and 5 g of polyethylene glycol 4000/l, together with one of the following (mmol/l): mannitol (60) (solution 1), mannitol (30) and glucose (30) (solution 2), glucose (60) (solution 3) or glucose (120) (solution 4). Osmolality of the solutions was 290 (1, 2 and 3) or 350 mmol/kg (4). Preliminary studies had established that [³H]polyethylene glycol 4000 was a satisfactory non-absorbed marker and that a steady state was reached after 40 min of continuous perfusion. The solutions were studied in order of increasing glucose concentration. After a 40 min equilibration period for each perfused solution, three consecutive 10 min samples were collected from proximal and distal sampling sites. These collections were staggered by 5 min, the transit time through the test segment [2]. From two piglets, 1–5 ml portions were stored at −30°C for later determination of lactate and bile-salt concentrations and the remainder held at 4°C for analysis of [³H]polyethylene glycol, glucose and Na⁺ within 15 h. Preliminary studies had shown stability of glucose concentrations in samples stored at 4°C for up to 24 h. Stools and urine were examined for reducing substances by Clinistest (Ames Co., Elkhart, IN, U.S.A.) tablets during the experiments. At the end of the study each piglet was killed by an overdose of thiopental sodium and the correct location of the perfusion tube confirmed.

Sodium was measured by flame spectrophotometry, osmolality by freezing-point depression, glucose by the glucose oxidase method [8], lactate with lactate dehydrogenase (Sigma Bulletin 824-UU), [³H]polyethylene glycol 4000 by β-scintillation spectrometry and bile salts by thin-layer chromatography [9].

Experiments in vitro

Twenty matched-fed litter mates were studied at 14–16 days of age: 10 were controls and 10 had severe diarrhoea, having been infected with transmissible gastroenteritis virus 40 h previously. Steady-state unidirectional mucosa-to-serosa and serosa-to-mucosa fluxes of 3-O-methyl-D-glucose were measured in four adjacent segments of stripped jejunal mucosa, mounted in Ussing short-circuited chambers. Both sides of the tissue, 1–29 cm² in area, were exposed to 15 ml of circulating oxygenated Krebs' buffer containing 3-O-methyl-D-glucose (20 mmol/l) at 37°C [6]. To measure unidirectional fluxes of the sugar, 10 µCi of d-3-O-[methyl-³H]glucose was added to either the mucosal or serosal buffer. Samples (1 ml) were taken from both sides of the chambers for radioactivity counting at 10 min intervals during two 30 min study periods, each preceded by 15 min equilibration. During the second study period, 3-O-methyl-D-glucose fluxes were measured in the presence of phlorizin (0.5 mmol/l), placed on the mucosal side of the tissue. Steady-state conditions were confirmed by comparing individual 10 min fluxes. Preliminary experiments done under identical conditions had shown stable tissue transport and electrical properties for more than 90 min in piglet jejunum. Having assessed piglet jejunum in the presence of phlorizin (0.005, 0.01, 0.05 and 0.10 mmol/l) we found maximal inhibition of net 3-O-methyl-D-glucose transport by 0.05 mmol/l. An adjacent segment of jejunal mucosa was also taken from each pig, fixed in Bouin's solution, sectioned and stained by the periodic acid-Schiff (PAS) technique for light microscopic study.

Standard formulae were used to calculate net fluxes of glucose, water and Na⁺ from perfusion data in vivo [10, 11]. For calculating unidirectional 3-O-methyl-D-glucose fluxes in vitro we modified the formula of Schultz & Zalusky [12], since both serosal and mucosal sides were sampled. The paired t-test was used to compare data in vivo and Student's t-test to compare data in vitro.

Results

Perfusion studies (Table 1)

Glucose absorption in the jejunum of acutely ill piglets was less than in control and convalescent
Glucose transport in viral enteritis

TABLE 1. Net jejunal transport of glucose, sodium and water in response to increasing perfusate concentrations of glucose in piglets studied before, 40 and 144 h after transmissible gastroenteritis infection

Results are expressed as means ± SEM; negative results indicate net accumulation in the lumen. Perfusion solutions contained (mmol/l): NaCl (115), chloride (110) and potassium bicarbonate (10). The number of animals studied are given in parentheses.

Significance of differences: *P < 0.05 compared with control piglets; **P < 0.001 compared with control piglets.

<table>
<thead>
<tr>
<th>Glucose perfusate concn. (mmol/l)</th>
<th>Net glucose flux (ml h⁻¹ cm⁻²)</th>
<th>Net Na⁺ flux (ml h⁻¹ cm⁻²)</th>
<th>Net water flux (ml h⁻¹ cm⁻²)</th>
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<tr>
<td></td>
<td>Control 0 h 40 h 144 h</td>
<td>Control 0 h 40 h 144 h</td>
<td>Control 0 h 40 h 144 h</td>
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<td>0</td>
<td>(8) 3 (8) 3 (8)</td>
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<tr>
<td></td>
<td>-0.131 -0.122 -0.099</td>
<td>-0.325 -0.333 -0.361</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>±0.030 ±0.019 ±0.045</td>
<td>±0.221 ±0.318 ±0.082</td>
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</tr>
<tr>
<td></td>
<td>±0.024*</td>
<td>±0.016*</td>
<td></td>
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<tr>
<td>60</td>
<td>±0.016* ±0.004 ±0.005</td>
<td>±0.045 ±0.061 ±0.026</td>
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<tr>
<td>120</td>
<td>±0.017* ±0.018</td>
<td>±0.017* ±0.018</td>
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TABLE 2. 3-O-Methyl-D-glucose fluxes (μmol h⁻¹ cm⁻²) in short-circuited jejunal mucosa from control and 40 h transmissible gastroenteritis infected piglets with and without phlorizin

Results are expressed as means ± SEM. The number of animals studied are given in parentheses.

Significance of differences: *P < 0.05 compared with control piglets; **P < 0.001 compared with control piglets; ††P < 0.005 compared with basal conditions; †††P < 0.001 compared with basal conditions.

<table>
<thead>
<tr>
<th>3-O-Methyl-d-glucose flux</th>
<th>Mucosa-to-serosa</th>
<th>Serosa-to-mucosa</th>
<th>Net</th>
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<tr>
<td>Basal conditions</td>
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<tr>
<td>Control (10)</td>
<td>0.969 ± 0.110</td>
<td>0.517 ± 0.036</td>
<td>0.454 ± 0.080</td>
</tr>
<tr>
<td>Transmissible gastroenteritis (10)</td>
<td>0.272 ± 0.022**</td>
<td>0.302 ± 0.017**</td>
<td>0.030 ± 0.020**</td>
</tr>
<tr>
<td>Phlorizin (0.05 mmol/l)</td>
<td></td>
<td></td>
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<tr>
<td>Control (6)</td>
<td>0.434 ± 0.030†</td>
<td>0.475 ± 0.030</td>
<td>-0.041 ± 0.027††</td>
</tr>
<tr>
<td>Transmissible gastroenteritis (8)</td>
<td>0.313 ± 0.041*</td>
<td>0.361 ± 0.038*</td>
<td>-0.049 ± 0.013</td>
</tr>
</tbody>
</table>

animals at all three perfusate glucose concentrations (P < 0.01). When the animals were studied during the control period and at 144 h, but not at 40 h, the increase of glucose concentrations in the perfusate progressively increased net absorption of glucose from the lumen (P < 0.05). When solution 1, containing no glucose, was perfused, net secretion of Na⁺ and water was observed during all three phases of the study; Na⁺ and water absorption was progressively enhanced by solutions 2, 3 and 4 during the control and convalescent phases, but not during the acute phase when these responses were greatly diminished and water fluxes remained secretory. At 40 h, a significant decrease in net Na⁺ and water secretion was observed when the perfusate glucose concentration was increased up to 60 mmol/l (solution 3), but no further improvement occurred at 120 mmol/l (solution 4). In the course of the perfusion studies stool and urine samples contained no reducing substances, samples of intestinal juice taken from the distal site contained negligible lactate concentrations, and concentrations of conjugated and unconjugated bile salts did not differ between groups.

Transport of 3-O-methyl-D-glucose in Ussing chambers (Table 2)

Mucosa-to-serosa and serosa-to-mucosa 3-O-methyl-D-glucose fluxes were greatly and significantly diminished (P < 0.001) in jejunal epithelium taken from piglets when diarrhoea was severe, 40 h after infection, compared with fluxes in tissue from control animals. Studied in the absence of an electrochemical gradient, mean net 3-O-methyl-D-glucose flux did not significantly differ from zero, and was significantly less than the absorptive net flux in control tissue (P < 0.001). The addition of phlorizin to control tissue in the second study period reduced unidirectional fluxes and abolished net absorption (P < 0.001),
FIG. 1. Photomicrographs of proximal jejunal mucosa from 16-day-old piglets. PAS stain (×40). (a) Control pig showing villi, crypts and regular columnar epithelium. (b) Pig 40 h after transmissible gastroenteritis virus infection showing short villi, deep crypts and cellular density in lamina propria compared with control animals. Surface epithelium is regular and columnar.
but in epithelium from the 40 h animals where net transport was already zero, no significant change in unidirectional or net 3-\textit{O}-methyl-D-glucose flux occurred. Unidirectional glucose fluxes in transmissible gastroenteritis piglets remained less than those in control animals in the presence of phlorizin ($P < 0.05$).

Photomicrographs of typical jejunal mucosa from an infected and a control animal are shown in Fig. 1. In the 16-day-old pig, 40 h after receiving transmissible gastroenteritis virus, the villi were intact, but relatively short, and the crypts relatively deep. The villus enterocytes were regular and columnar.

**Discussion**

Glucose absorption is severely impaired in the jejunum of piglets with acute transmissible gastroenteritis. Our marker perfusion experiments demonstrated that net glucose absorption was significantly diminished 40 h after experimental infection. Furthermore absorption failed to increase as perfusate glucose increased from 30 to 120 mmol/l, although concentrations of 30 mmol/l or more favoured diffusion of glucose from the lumen to the extracellular fluid. These findings are in marked contrast with those from the control period when absorption increased progressively as perfusate glucose concentrations rose. The Ussing chamber data suggest that abnormalities occurred in both passive and active glucose transport. Markedly decreased unidirectional 3-\textit{O}-methyl-D-glucose fluxes in the 40 h tissue indicate decreased passive movement of the sugar across the jejunal epithelium. Active transport was undoubtedly defective too, since net absorption was not detectable in transmissible gastroenteritis tissue. Phlorizin, a potent inhibitor of glucose transport at the brush border [13], had no effect on 3-\textit{O}-methyl-D-glucose flux, a finding that indicates a lack of effective jejunal brush-border sites for glucose transport in acute transmissible gastroenteritis. Since intraluminal lactic acid levels did not rise during the perfusion studies, and the Ussing chamber experiment utilized a non-metabolizable sugar, we found no evidence to suggest that these abnormal findings could be attributed to an effect of the infection on luminal or mucosal glucose metabolism.

The diminished response of Na$^+$ transport \textit{in vivo} to a rising concentration of perfusate glucose is in keeping with the current glucose transport data and with previous studies \textit{in vitro} in our laboratory showing that glucose-stimulated Na$^+$ transport was defective in acute transmissible gastroenteritis [6]. Glucose up to 60 mmol/l did decrease net Na$^+$ and water secretion, but no further changes were seen at 120 mmol/l, indicating saturation of available transport mechanisms. In fact, net secretion of Na$^+$ and water persisted in the presence of glucose. In convalescent animals glucose absorption had recovered, but persisting significant abnormalities of Na$^+$ and water flux suggest that recovery was not complete at 144 h.

In infants with acquired monosaccharide intolerance glucose malabsorption has been attributed to loss of intestinal surface area [14]. The present data and studies done in our laboratory and elsewhere have shown a microscopic mucosal lesion with varying degrees of shortening of villi in acute transmissible gastroenteritis [15, 16]. Impaired glucose transport cannot be attributed to direct viral damage to the epithelium since previous studies have shown that the virus has been shed by the 40 h stage of the disease [15, 16] and neither reinfection by transmissible gastroenteritis virus nor secondary bacterial contamination of the gut lumen have been demonstrated [2, 16]. Additional evidence against bacterial contamination comes from the present finding of normal bile-salt patterns in intestinal juices from the 40 h piglets. In acute transmissible gastroenteritis the villi are populated by a relatively undifferentiated crypt-type epithelium [5] and glucose transport properties of this undifferentiated tissue are known to differ from those of a mature epithelium [17]. Also studies of normal rat crypt cells in suspension have shown a lack of the glucose-stimulated Na$^+$ transport normally present in mature villus cells, suggesting a defect in the brush-border carrier shared by Na$^+$ and glucose [18]. Whether transmissible gastroenteritis infection and the consequent failure of epithelial differentiation affects other sites of active and passive epithelial glucose transport remains to be determined.

In mild cases of transmissible gastroenteritis, little or no diarrhoea may occur. Presumably, when a relatively limited segment of proximal small bowel is affected, the uninvolved distal portions can compensate for the functional derangements occurring 'up-stream'. However, transmissible gastroenteritis virus can invade the entire length of the small bowel, particularly in very young piglets [16]. The diarrhoea of this invasive enteritis will be influenced by the extent to which the ileum is affected, in addition to the actual severity of the epithelial transport defects. Clearly, impaired glucose absorption is an important determinant of the volume of stool presented to the distal portion of the small bowel and,
therefore, a significant determinant of the severity of diarrhoea. Some infants with undefined infectious diarrhoea have impaired glucose transport in the small bowel [19]. Our studies were conducted in piglets infected with swine virus, but some comment on the clinical relevance of these animal studies is appropriate. The major known enteric infection of human infants, human rotavirus enteritis, although caused by a different agent, bears remarkable similarities to transmissible gastroenteritis, particularly with regard to the site of disease and pathophysiology of the resultant diarrhoea [3,4]. Identification of a defect in glucose transport in acute viral enteritis emphasizes the difference between enterotoxigenic diarrhoeas, in which orally given glucose may reverse Na+ and water secretion [20], and acute viral diarrhoea. Although some capacity to absorb glucose can persist in transmissible gastroenteritis, we could not demonstrate reversal of water and Na+ secretion in response to glucose. In designing oral-treatment strategies for infants with acute viral diarrhoea advantage must be taken of any remaining intestinal capacity for glucose absorption, but, clearly, that absorptive capacity might easily be overwhelmed. Bangladeshi children with rotavirus diarrhoea coped with oral sugar/electrolyte solutions containing sucrose or glucose (111 mmol/l), but a high proportion of these patients had excessive sugar in their stools [21]. While awaiting further therapeutic trials, we wish to emphasize the potential hazard of undiluted proprietary beverages such as fruit juices and soft drinks in the oral rehydration of young infants with severe viral diarrhoea. These products may contain sugars in concentrations sufficiently high [22] to overwhelm the limited absorptive capacity of a child’s gut during an acute invasive viral enteritis.

Acknowledgments

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