Effect of cholera toxin on glutamine metabolism and transport in rabbit ileum

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Abely, M., P. Dallet, M. Boisset, and J. F. Desjeux. Effect of cholera toxin on glutamine metabolism and transport in rabbit ileum. Am J Physiol Gastrointest Liver Physiol 278: G789–G796, 2000.—The aim of the present study was to evaluate the effect of cholera toxin on energy balance from intestinal glutamine metabolism and oxidation, glutamine-dependent sodium absorption, and cholera toxin-dependent ion flux. Cholera toxin-stimulated sodium and L-glutamine ileal transport and metabolism were studied in Ussing chambers. Glutamine (10 mM) transport and metabolism were simultaneously studied using 14C flux and HPLC. In the same tissues, the flux of each amino acid was studied by HPLC, and glutamine metabolism and oxidation were studied by the determination of amino acid specific activity and 14CO2 production. In control tissues, glutamine-stimulated sodium absorption and was mainly oxidized. The transepithelial flux of intact glutamine represented 45% of glutamine flux across the luminal membrane. The other metabolites were glutamate and, to a lesser degree, citrulline, ornithine, and proline. Cholera toxin did not alter glutamine-stimulated sodium absorption, L-glutamine oxidation, transport, and metabolism.

In conclusion, the present results indicate that cholera toxin does alter glutamine intestinal function and metabolism. In addition, ~95% of the energy provided by glutamine oxidation remains available to the enterocyte.

electrolyte transport; ATP balance; intestinal mucosa; P5C pathway; rehydration solution

Cholera is often regarded by physiologists as an interesting model for studying the response of intestinal mucosa to a well-defined toxin, cholera toxin (CT). The water and electrolyte electrolyte secretion that result from CT challenge have been extensively studied (4). In experimental and human cholera, the relationship between electrolyte and water fluxes across the intestine remains identical to control conditions (5, 32). In addition, the findings that the function of the glucose-Na+ cotransporter at the luminal membrane of the epithelial layers is enhanced by CT (24, 31) provided an interesting explanation for the clinical efficacy of the oral rehydration solution (ORS) recommended by the World Health Organization (35). The World Health Organization ORS contains a concentration of electrolytes approximately equivalent to that lost in stools of patients with severe cholera (90 mmol/l Na+ and 20 mmol/l K+) and 110 mmol/l glucose to stimulate intestinal Na+ absorption (9, 36).

However, cholera remains a widespread and life-threatening disease for several reasons, including the lack of an effective immunization program and a frequent association between cholera and poor nutritional status (36). The treatment of patients with dehydration and malnutrition is particularly complex because the acute metabolic alterations of acute dehydration are superimposed on the long-lasting metabolic disturbances of chronic malnutrition. The present recommendation is to first rehydrate with an ORS, called ReSoMal, that takes into account the amount of water and electrolyte lost in stools and the chronic deficit in K+, Mg2+, and micronutrients and then to feed the patient as soon as possible (36). However, when feeding is initiated, nutrient intestinal absorption may not be optimal as a consequence of malnutrition and partial rehydration (2, 3). At the rehydration phase, except for glucose used to stimulate rehydration, no other nutrient has proved to be functionally effective and useful in the intestinal functions.

Glutamine may be considered a possible candidate to add to ORS for several reasons: 1) It stimulates intestinal Na+ absorption, as demonstrated in an in vitro isolated intestine model (7, 23, 26, 28) and in vivo intestinal perfusion in adult cholera patients in Bangladesh (32); 2) It is also an important source of energy for the intestinal mucosa in animals and humans (6, 30, 33); and 3) It is involved in many important metabolic processes that may favor intestinal repair in malnutrition (15). However, it is not known how much of the energy that glutamine provides to the intestine is used for the stimulation of electrolyte transport and its own metabolism. Also, it is not known whether CT that stimulates electrolyte secretion also alters glutamine metabolism. In addition, most metabolic studies of glutamine are not conducted under conditions that are relevant to a situation where glutamine is used for its functional properties in intestinal Na+ absorption.

The aim of the present study was therefore to evaluate the effect of CT on energy balance from intestinal glutamine metabolism and oxidation, glutamine-dependent Na+ absorption, and CT-dependent ion flux alteration. To do so, we coupled transport studies in Ussing chambers that have proven effective in understanding nutrient and electrolyte intestinal transport alteration due to CT and HPLC with isotopic tracer methods to identify the glutamine metabolites in isolated rabbit ileum mucosa. Thus the aim was not to duplicate

*The first and second authors contributed equally to this work.

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previous studies of the effect of glutamine on Na$^+$ absorption or glutamine metabolism (7, 23, 26, 28, 32, 34). Rather, we wanted to link these approaches to previous studies of the effect of glutamine on Na$^+$ absorption. We were interested in linking these approaches to previous studies of the effect of glutamine on Na$^+$ absorption and Cl$^-$ secretion.

MATERIALS AND METHODS

Animals. Before death, healthy male weanling New Zealand White rabbits (établissement CEGAV, Saint Mars d’Egrenne, France) were fed with 13% protein standard formula (Lapin entretien 112; UAR, Epinay sur Orge, France). Rabbit weight was 2.15 ± 0.12 kg. They were fasted 18 h before anesthesia with 1 ml/kg of 3% (wt/vol) pentobarbital sodium. Small bowel was washed with 37°C Ringer solution. Rabbit solution (9 ml) was introduced in each loop, and 20 µg of CT (Sigma, Saint Louis, MO) was added in one of them. After 3 h, rabbits were killed using pentobarbital (2 ml/kg).

Electrical measurements. For each animal, measurements were assessed in 4–8 controls and 4–8 cholera tissues. Ileal mucosa was mounted between two half-chambers, exposing an area of 1.13 cm$^2$, and bathed at 37°C with 12 ml of oxygenated Ringer buffer containing (in mM) 140 Na$^+$, 5.2 K$^+$, 1.2 Ca$^{2+}$, 1.2 Mg$^{2+}$, 120 Cl$^-$, 25 HCO$_3^-$, 2.4 HPO$_4^{2-}$ and 0.4 H$_2$PO$_4^-$. After 30 min, 10 mM L-glutamine was added to the mucosal side, with equimolar mannitol added to the serosal side. Potential difference (PD) across the tissue was measured by calomel electrodes in saturated KCl using agar bridges positioned near the surface of the tissue. By Ag-AgCl electrodes connected to the solution via agar bridges, the tissue was continuously short-circuited with automatic voltage clamps (World Precision Instruments, Sarasota, FL) that compensated for the fluid resistance. Ileal sheets were mounted in the chambers within 30 min of rabbit death and continuously short-circuited, except for a 10-s interval every 15 min, when the open-circuit PD was measured. Conductance was calculated from change in current following an imposed voltage using Ohm’s law. An increase in short-circuit current (Isc) corresponds to cation transepithelial absorption or anion secretion.

I on flux measurements. The transepithelial unidirectional Na$^+$ and Cl$^-$ fluxes from mucosa to serosa and from serosa to mucosa were determined in six rabbits and were both studied in the same period. For each animal, measurements were assessed in eight control and eight cholera tissues. The stability of the electrical parameters was checked for at least 15 min; 1 µCi of $^{22}$Na and 2 µCi of $^{36}$Cl were then added to the appropriate reservoir, and a 10-min equilibration period was allowed to elapse before unidirectional fluxes were determined for five 10-min periods. After 60 min, 10 mM L-glutamine was introduced into the mucosal compartment and 10 mM mannitol was introduced into the serosal side. Electrical parameters and unidirectional fluxes were determined for three 20-min periods. The $^{22}$Na fluxes were measured using a gamma counter (Kontron), and the $^{36}$Cl fluxes were measured using a liquid scintillation spectrometer (SL 4000 IN Intertechnique PG 4000).

Glutamine transport and metabolism. Associated with 10 mM L-glutamine (Sigma), 2 µCi glutamine uniformly labeled with $^{14}$C (NEN, Boston, MA) was added to the mucosal compartment. The $^{14}$C flux across the epithelium representing the overall flux of $[^{14}\text{C}]]$glutamine and $^{14}$C metabolites was therefore expressed as glutamine equivalent flux. Samples were taken every 30 min in the serosal compartment. The transfer of radioactivity was measured using a liquid scintillation spectrometer. For each sample, two 10-min countings were done. The mean of these two countings was used for flux calculation using the counts per minute accumulation method, taking into account a constant quenching (23).

The glutamine oxidative metabolism produced CO$_2$ that was not included in the glutamine equivalent flux. The oxidative metabolism was therefore estimated by trapping CO$_2$ produced in the two compartments in a series of two tubes containing 1 M NaOH (5 ml). Radioactivity was estimated using the liquid scintillation spectrometer (1-ml sample associated with 4-ml scintillator). The $^{14}$CO$_2$ production was calculated from the $^{14}$C counted in NaOH and the specific activity of $[^{14}\text{C}]$glutamine, uniformly labeled on its five carbons, as checked by HPLC coupled with liquid scintillation.

The fluxes of glutamine metabolites were assessed using a liquid chromatograph with ultraviolet detector (Shimadzu, Kyoto, Japan) coupled with a fraction collector (Foxy 200 ISCO; Isco, Lincoln, NE). Because glutamine and metabolites (glutamate, alanine, ornithine, citrulline, and proline) do not absorb in ultraviolet or in visible light, they were covalently bound to a chromophore. The derivatization reagent was dimethylaminoazobenzene sulfonyl chloride (DABSYL-Cl) with maximal absorbency at 436 nm. Samples (100 µl) were reacted for 10 min at 70°C with 50 µl NaHCO$_3$ (150 mM, pH 8.6) and 150 µl DABSYL-Cl (8 mM; Fluka, Buchs, Switzerland) in acetone. Dilution was made with 300 µl KH$_2$PO$_4$ (12.5 mM, pH 3) and ethanol (50/50 vol/vol). Fifty microliters were injected (Fig. 1). The column was a C8 Kromasil type (250 mm × 45 mm, 5 µm particle size; Eka Chemicals, Böhus, Sweden). Oven temperature was 40°C. Mobile phase was composed of 12.5 mM KH$_2$PO$_4$, pH 3 (solvent A) and acetonitrile/isoamyl alcohol (v/v, 1/1) (solvent B). Flow rate was 1.3 ml/min. The gradient was 10% of solvent B at 0 min, 30% of solvent B at 2 min, 30% of solvent B at 10 min, 45% of solvent B at 20 min, 55% of solvent B at 40 min, 60% of solvent B at 44 min, 70% of solvent B at 47 min, and 100% of solvent B at 48 min. Run time was 53 min. No other component was eluted at the retention time of glutamine, Cit, Arg, and Orn (8) were (in µM) 4.5, 114.4, 2.3, 10.3, 30.3, 18.6, 3.2, and 4.6, respectively. Retention times of Arg, Gin, Cit, Asp, Glu, Ala, Pro, and Orn (in min) 18.15, 21.58, 22.37, 24.33, 24.90, 29.08, 32.77, and 46.75, respectively.
Table 1. Effect of glutamine on electrical parameters in control and cholera toxin-treated rabbit ileum

<table>
<thead>
<tr>
<th></th>
<th>Ringer</th>
<th>Ringer + Glutamine</th>
<th>ΔGlutamine – Ringer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>PD, mV</td>
<td>I_sc, µEq·h⁻¹·cm⁻²</td>
<td>G, mS/cm</td>
</tr>
<tr>
<td>Control</td>
<td>2 ± 0.16</td>
<td>1.56 ± 0.1</td>
<td>17.05 ± 0.46</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>5.5 ± 0.16</td>
<td>2.16 ± 0.16</td>
<td>18.9 ± 2.3</td>
</tr>
<tr>
<td>P</td>
<td>0.0002</td>
<td>0.004</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 rabbits. Number of studied pieces of ileum was 4–8 taken from control or cholera toxin-treated ileal loops. PD, transepithelial potential difference; I_sc, short-circuit current; G, transepithelial conductance. After basal data obtained in Ringer solution, L-glutamine (10 mM) was added to mucosal side and mannitol (10 mM) to serosal side of ileal tissues mounted in Ussing chambers. ΔGlutamine – Ringer is the difference between data before and after glutamine addition. P values correspond to Tukey’s test performed after general linear model ANOVA.

citrulline, glutamate, alanine, proline, or ornithine. To verify repeatability, a mixture of the six amino acids was derivatized 10 times, with a relative standard deviation range from 2.1 to 4.0%. The method was linear in a range of 20–200% of the concentration observed for a flux of 1 µmol·h⁻¹·cm⁻² (glutamine equivalent). Recovery ranged from 98.4 to 101.4%. The fraction collector was connected directly to the ultraviolet detector output. Beta counting was done on the collected fraction using liquid scintillation spectrometry.

Statistical analysis. Data were analyzed using SAS software (SAS, Cary, NC). Data were summarized as means ± SE with n = number of rabbits. The P values correspond to a general linear model analysis.

RESULTS

Control tissue. In control tissues, addition of 10 mM glutamine to the mucosal compartment was followed by an immediate and steady rise in I_sc (2.2 ± 0.22 µmol·h⁻¹·cm⁻²; Table 1). After 2 h, further addition of 10 mM glucose was again followed by an additional rise in I_sc (2.1 ± 0.59 µmol·h⁻¹·cm⁻²; Table 2). After both substrate additions, transepithelial conductance increased by 38 and 26% for glutamine and glucose, respectively.

Glutamine equivalent flux reached a steady state at 30 min and remained stable at 0.88 ± 0.16 µmol·h⁻¹·cm⁻² (Table 3). The total amount of intact glutamine entering across the luminal membrane could be estimated by the sum of [¹⁴C]glutamine equivalent flux and [¹⁴C]CO₂ flux produced by the tissue divided by 5 to take into account the five carbon atoms in one molecule of glutamine. Thus the intact glutamine flux entering the cell from the mucosal solution was 1.31 µmol·h⁻¹·cm⁻², of which 34% was oxidized into CO₂.

The transepithelial fluxes of intact glutamine and its metabolites were measured by HPLC. Figure 1 shows a typical recording of a serosal sample. Intact glutamine was identified and quantified, yielding a flux of glutamine appearing in the serosal compartment of 48% of the flux of the glutamine entering the tissue across the luminal membrane (Table 3). Each amine metabolite was clearly identified, i.e., glutamate, alanine, citrulline, proline, and ornithine (Fig. 1). Accordingly, the fluxes of metabolites appearing in the serosal compartment were measured. The glutamate and alanine fluxes were 15 and 11%, respectively, of the flux of intact glutamine entering the cell. In contrast, the amount of ornithine, citrulline, and proline was negligible compared with glutamine flux. No aspartate was detected in the serosal fluid. Thus the flux of the substrates appearing in the serosal compartment accounted for 108% of the intact glutamine flux entering the cell from the luminal compartment.

The 8% excess of substrates entering the serosal compartment may have been related to endogenous release of substrates from the cell in absence of exogenous glutamine. However, before glutamine addition in the mucosal compartment or in the absence of glutamine during 120 min, none of these metabolites were detected by HPLC except for small quantities of glutamate. In addition, alanine that was present in the serosal compartment after 30 min disappeared after 120 min.

Another explanation may be related to the complexity of the carbon recycling in the enterocyte. We thus examined the specific activity of the different collected fractions. The specific activity for glutamine, glutamate, and alanine was 98, 33, and 11%, respectively. Thus the [¹⁴C]glutamate and [¹⁴C]alanine fluxes entering the serosal compartment were 5 and 1.2%, respect-

Table 2. Effect of glucose on electrical parameters in control and cholera toxin-treated rabbit ileum previously stimulated by glutamine

<table>
<thead>
<tr>
<th></th>
<th>Ringer + Glutamine + Glucose</th>
<th>ΔGlucose – Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>PD, mV</td>
<td>G, mS/cm</td>
</tr>
<tr>
<td>Control</td>
<td>4.2 ± 0.34</td>
<td>29.77 ± 2.48</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>6.74 ± 0.37</td>
<td>42.16 ± 4.47</td>
</tr>
<tr>
<td>P</td>
<td>0.0079</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 rabbits. α-D-Glucose (10 mM) was added on mucosal side, and electrical parameters were recorded 15 min later. ΔGlucose – glutamine is the difference between data before and after glucose addition.
tively, of the intact glutamine entering the cell from the mucosal compartment.

In summary, 88% of the 14C-labeled glutamine entering the cell from the mucosal compartment was found and identified in the serosal compartment. For the other metabolites, the specific activity determination was considered unreliable considering the very low amino acid concentration. In addition, lactate and pyruvate were not detected in our conditions.

After addition of glutamine, net Na⁺ flux increased by 1.37 ± 0.51 µmol·h⁻¹·cm⁻² (Table 4). The increased net Cl⁻ flux (0.78 ± 0.51 µmol·h⁻¹·cm⁻²) was interpreted as a stimulation of a neutral NaCl flux (0.78 µmol·h⁻¹·cm⁻²), and the difference between net Na⁺ flux and net Cl⁻ flux was interpreted as a stimulation of an electrogenic Na⁺ flux (0.59 µmol·h⁻¹·cm⁻²). Accordingly, the residual electrogenic flux (Iₑₑ – electrogenic Na⁺ absorption) was 1.57 µmol·h⁻¹·cm⁻². In addition, all the ionic unidirectional fluxes increased in relation to increased conductance. The electrical parameters observed in the second set of experiments (Table 3) were similar to those measured in the first set of experiments (Table 1).

Effect of CT. In CT-treated loops, water secretion was observed after 3 h (−7.0 ± 0.72 ml) and water absorption was observed in control Ringer loops (4.1 ± 0.94 ml). When loops were mounted in Ussing chambers, Iₑₑ was significantly higher (P < 0.004) in CT than control tissue (Table 1). The conductance was essentially identical in both conditions. Glutamine equally stimulated Iₑₑ in CT and in control tissues, but conductance increase was significantly more pronounced in CT than control tissues (P < 0.001). After 2 h, further addition of 10 mM glucose was again followed by an additional rise in Iₑₑ (2.1 ± 0.17 µmol·h⁻¹·cm⁻²). After both substrate additions, transepithelial conductance increased by 83 and 39% for glutamine and glucose, respectively (Tables 1 and 2).

In CT tissues, no statistical difference compared with control tissues was observed in glutamine equivalent flux from mucosa to serosa, intact glutamine flux across the luminal membrane, 14CO₂ production, and glutamine and metabolites appearing in the serosal compartment. The only observed statistical difference was an increase in citrulline flux (Table 3), for which we have no explanation.

Glutamine equivalent flux reached a steady state at 30 min and remained stable at 0.9 ± 0.19 µmol·h⁻¹·cm⁻² (Table 3). The intact glutamine flux entering the cell from the mucosal solution was 1.42 µmol·h⁻¹·cm⁻², of which 30% was oxidized in CO₂. Thus the intact glutamine transepithelial flux from mucosa to serosa was 50% of the flux of the glutamine entering the tissue. The glutamate and alanine fluxes were 8 and 11%, respectively, of the flux of intact glutamine entering the cell.

CT did not modify the glutamine effect on net Na⁺ flux (1.22 ± 0.42 µmol·h⁻¹·cm⁻²) and net Cl⁻ flux (0.81 ± 0.68 µmol·h⁻¹·cm⁻²), neutral NaCl flux (0.81 µmol·h⁻¹·cm⁻²), electrogenic Na⁺ flux (0.41 µmol·h⁻¹·cm⁻²), and residual flux (1.27 µmol·h⁻¹·cm⁻²) (Table 4). It increased all the Na⁺ and Cl⁻ unidirectional fluxes (Table 4) and the transepithelial conductance (14.52 ± 2.75 mS/cm²).

DISCUSSION

The present study confirms that glutamine stimulates electrolyte absorption in control and CT-treated tissues. In both conditions, glucose displays an additional effect on electrolyte absorption. In addition, the present results indicate that CT does not alter effects of

Table 3. Glutamine and glutamine metabolite fluxes in control and cholera toxin-treated rabbit ileum

<table>
<thead>
<tr>
<th>Ileum</th>
<th>J_Glu Equivalent, µmol·h⁻¹·cm⁻²</th>
<th>J_Glu, µmol·h⁻¹·cm⁻²</th>
<th>J_GluCO₂, µmol·h⁻¹·cm⁻²</th>
<th>J_Glu, µmol·h⁻¹·cm⁻²</th>
<th>J_Glu, µmol·h⁻¹·cm⁻²</th>
<th>J_Alta, µmol·h⁻¹·cm⁻²</th>
<th>J_Cla, µmol·h⁻¹·cm⁻²</th>
<th>J_Glu, µmol·h⁻¹·cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.88 ± 0.16</td>
<td>0.63 ± 0.18</td>
<td>2.17 ± 0.48</td>
<td>0.2 ± 0.05</td>
<td>0.15 ± 0.03</td>
<td>15.36 ± 5.48</td>
<td>9.4 ± 1.71</td>
<td>4.28 ± 3.94</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>0.9 ± 0.19</td>
<td>0.72 ± 0.2</td>
<td>2.6 ± 0.35</td>
<td>0.12 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>10.07 ± 2.8</td>
<td>18.8 ± 4.8</td>
<td>5.64 ± 5.26</td>
</tr>
<tr>
<td>P</td>
<td>0.93</td>
<td>0.74</td>
<td>0.47</td>
<td>0.06</td>
<td>0.9</td>
<td>0.4</td>
<td>0.04</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 rabbits. All fluxes (J) are unidirectional mucosa-to-serosa fluxes, except for 14CO₂, which was collected on both sides of the tissue. Fluxes were measured 120 min after mucosal addition of 10 mM L-[14C]glutamine. [14C]glutamine equivalent flux (J_Glu Equivalent) was measured as cumulative amount of 14C present in serosal compartment.

Table 4. Effect of glutamine on Na⁺ and Cl⁻ fluxes and electrical parameters in control and cholera toxin-treated rabbit ileum

<table>
<thead>
<tr>
<th>Ileum</th>
<th>J_Na⁺ , µmol·h⁻¹·cm⁻²</th>
<th>J_Cla , µmol·h⁻¹·cm⁻²</th>
<th>J_Na⁺ , µmol·h⁻¹·cm⁻²</th>
<th>J_Cla , µmol·h⁻¹·cm⁻²</th>
<th>J_Na⁺ , µmol·h⁻¹·cm⁻²</th>
<th>J_Cla , µmol·h⁻¹·cm⁻²</th>
<th>Iₑₑ , µEq·h⁻¹·cm⁻²</th>
<th>E , mV/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.89 ± 0.89</td>
<td>1.52 ± 0.64</td>
<td>1.37 ± 0.51</td>
<td>3.05 ± 0.71</td>
<td>2.27 ± 0.31</td>
<td>0.78 ± 0.51</td>
<td>2.16 ± 0.38</td>
<td>8.05 ± 3.21</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>5.14 ± 0.45</td>
<td>3.91 ± 0.58</td>
<td>1.22 ± 0.42</td>
<td>4.89 ± 0.98</td>
<td>4.08 ± 0.71</td>
<td>0.81 ± 0.68</td>
<td>1.68 ± 0.51</td>
<td>14.52 ± 2.75</td>
</tr>
<tr>
<td>P</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
<td>0.5</td>
<td>0.3</td>
<td>0.006</td>
<td></td>
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</table>

Values are means ± SE. In each of the 6 rabbits, 16 pieces of unstripped ileum (i.e., 8 control and 8 cholera toxin-treated) were mounted in Ussing chambers. J_Na⁺, mucosa-to-serosa Na⁺ flux; J_Cla, serosa-to-mucosa Cl⁻ flux; J_Na⁺, net Na⁺ flux; J_Cla, net Cl⁻ flux; J_Na⁺, serosa-to-mucosa Cl⁻ flux; J_Cla, mucosa-to-serosa Na⁺ flux; J_glu, glutamine flux.
Fig. 2. Transport and metabolism of glutamine in control rabbit ileum. Steady-state fluxes of intact glutamine, products of glutamine metabolism, and electrolytes were assessed as described in MATERIALS AND METHODS. The main assumptions for calculation of ATP production were: 1) 1.31 µmol·h⁻¹·cm⁻² ATP was synthesized from glutamine oxidation; 2) ATP production was complete, with a stoichiometry of 3 Na⁺:1K⁺:2Cl⁻:2 of synthesized glutamate, estimated by difference between glutamine−CO₂ production (2.2 µmol·h⁻¹·cm⁻²) and intact glutamine flux (0.63 µmol·h⁻¹·cm⁻²); 3) Na⁺ absorption was electrogenic in control conditions (Fig. 2), 1.31 µmol·h⁻¹·cm⁻² Na⁺ entered the enterocyte with 0.8 µmol·h⁻¹·cm⁻² Cl⁻ as electrogenic Na⁺ absorption; in CT (Fig. 3), 1.4 µmol·h⁻¹·cm⁻² glutamine entered with 0.8 µmol·h⁻¹·cm⁻² Cl⁻ as electrogenic Na⁺ absorption; in healthy and diarrheagenic Escherichia coli-infected rabbits and induced a 2-to-1 ratio. Rhoads et al. (23) demonstrated that glutamine enhanced sodium−K⁺−ATPase activity 3.5 times more electroneutral than electrogenic Na⁺ absorption in the jejunum of 1- to 3-wk-old piglets. In piglet rotavirus enteritis, glutamine stimulated equal amounts of electronegative and electroneutral Na⁺ absorption (28). In experimental Cryptosporidium infection in piglet (1), L-glutamine stimulated neutral NaCl absorption 1.3 times more than electrogenic Na⁺ absorption. The magnitude of stimulation of neutral NaCl absorption in the infected ileum exceeded that in control tissue, whereas electronegative Na⁺ absorption was smaller. Nath et al. (23) demonstrated that glutamine enhanced electronegative Na⁺ absorption in healthy and diarrheagenic Escherichia coli-infected rabbits and induced a small (10%) increase in the electrogenic absorption). Electroneutral NaCl absorption in infected rabbits. In summary, the relative magnitude of the two glutamine-stimulated Na⁺ absorptive processes varied from 0.10 to 3.5 (neutral/electrogenic Na⁺ absorption) according to animal species, age, or pathological conditions. In addition, in our experiments glutamine stimulated a large residual electronegative NaCl absorption. It may be related to the large CO₂ production (2.2 µmol·h⁻¹·cm⁻²) from glutamine metabolism. Whatever the final interpretation of the isotopic fluxes and electrical parameters, our results indicate that CT did not alter the net fluxes; it increased the conductance and the unidirectional fluxes.

For the calculation of energy expenditure related to glutamine-stimulated Na⁺ absorption, we used the following: in control conditions (Fig. 2), 1.31 µmol·h⁻¹·cm⁻² glutamine entered the enterocyte with 0.8 µmol·h⁻¹·cm⁻² Na⁺ as neutral flux and 0.59 µmol·h⁻¹·cm⁻² as electronegative Na⁺ flux; in CT (Fig. 3), 1.4 µmol·h⁻¹·cm⁻² glutamine entered with 0.8 µmol·h⁻¹·cm⁻² Na⁺ as neutral flux and 0.4 µmol·h⁻¹·cm⁻² as electronegative Na⁺ flux.

The ATP cost calculation was based on the observation that Na⁺−K⁺−ATPase is the main energy-driven transport process on which all electrolyte transport is dependent. The well-known stoichiometry of 3 Na⁺ expelled from the cell for 2 K⁺ entering at the expense of one ATP provides a reasonable estimate of energy used for transport. In control conditions, the presence of 10 mM glutamine at the luminal membrane was associated with an increase of 1.39 µmol·h⁻¹·cm⁻² Na⁺ absorption that hydrolyzed 0.46 µmol·h⁻¹·cm⁻² ATP (Fig. 2). In CT-treated intestine, 0.3 µmol·h⁻¹·cm⁻² Na⁺ entered the cell associated with 0.6 µmol·h⁻¹·cm⁻² Cl⁻ through basolateral Na⁺−K⁺−2Cl⁻ transport; it was then expelled through the Na⁺−K⁺−ATPase, which hydrolyzed an additional 0.1 µmol·h⁻¹·cm⁻² ATP.
From a methodological point of view, we did not intend to describe the different metabolic steps; rather, we clearly identified the oxidative pathway. Although measured exclusively in the serosal compartment, the Δ-pyrroline-5-carboxylate (P5C) pathway metabolites were identified in very small quantity. Most studies found such a metabolism, except one in rabbit ileum (23). The unexpected findings of that study may be explained by the methodology used, in which metabolism was measured by comparing [15N]glutamine and [14C]glutamine. However, the measurements were not performed on the same piece of intestine. Thus the variance due to the variable “rabbit” or “tissue” may have masked the difference related to metabolism.

It has previously been reported that the rate of glutamine metabolism and oxidation depends on the intraluminal glutamine concentration (20, 33). About 70% of the glutamine transported across the brush border was not metabolized in the intestine when the intraluminal glutamine concentration was 45 mM, but only 34% was transported intact to the serosal side when the concentration was 6 mM (33). Our results are in agreement with these results because 48% of glutamine was not metabolized when the intraluminal concentration was 10 mM. The reason for increased intact glutamine transport with increasing glutamine luminal concentration is to be found in the kinetic parameters of the metabolic and transport processes. In agreement with this hypothesis, we found that 0.33 ± 0.08 µmol·h⁻¹·cm⁻² of [14C]glutamine metabolites were present before intact glutamine was transported (Fig. 4). In addition, a transepithelial diffusional pathway is frequently observed with increasing luminal concentration (8). Thus it seems that for a luminal concentration as low as ~0.5–1 mM, the energy provided by glutamine has reached a maximal value. Above this threshold value, glutamine that is not metabolized is transported intact to the blood side.

We do not have quantitative determination of the concentration of glutamine in the human intestinal lumen after a meal or a glutamine-containing oral rehydration solution. However, human perfusion studies indicate that jejunal absorptive capacity of glutamine and glucose is essentially the same in control and cholera conditions (8, 22, 32). Direct measurement of glucose concentration in rat small intestinal lumen after a meal indicated a mean value of 0.2–24 mM; it ranged with time and small intestinal region from 0.2 mM to a maximum of 48 mM (11). Thus if glutamine luminal concentration varies in a similar range, the energy derived from glutamine in the intestinal cell may not be very different from what we measured using 10 mM glutamine.

For the energy produced by glutamine metabolism, we counted that one mole of ATP was used for each mole of glutamate produced. The production of glutamate was estimated to be 0.68 µmol·h⁻¹·cm⁻², assuming that all the difference between glutamine entering the

Fig. 3. Transport and metabolism of glutamine in cholera toxin rabbit ileum. Steady-state fluxes of intact glutamine, products of glutamine metabolism, and electrolyte transport were assessed as described for control rabbit ileum (Fig. 2), using the same assumptions for calculations. Cholera toxin stimulated an electrogenic Cl⁻ secretion without altering glutamine metabolism and transport. Thus ~5% of ATP produced from glutamine metabolism was used for glutamine and electrolyte transport (see DISCUSSION).

Fig. 4. Relationship between intact glutamine flux (J Gln) and glutamine equivalent flux (J [14C]Gln Equivalent) in rabbit ileum with without previous exposure to cholera toxin. Mucosal-to-serosal fluxes of intact glutamine and glutamine equivalent from control (●) and cholera toxin-treated (○) ileum were measured as described in MATERIALS AND METHODS. Equation of linear regression was J [14C]Gln Equivalent = (0.83 ± 0.09)·J Gln + (0.33 ± 0.08) (r = 0.92, P < 0.001).
cell (1.31 µmol·h⁻¹·cm⁻²) minus the glutamine found in the serosal compartment (0.63 µmol·h⁻¹·cm⁻²) has been deaminated to glutamate (Fig. 2).

The other metabolic pathway is the P5C synthetase pathway leading to ornithine, citrulline, and proline synthesis (34). In terms of energy consumption it may be regarded as negligible, because the metabolic flux is very low. However, the P5C pathway is functionally important, as exemplified by the report concerning two brothers with severe clinical symptoms related to P5C synthase deficiency (19).

Windmueller and Spaeth (34) demonstrated that [¹⁴C]glutamine synthesis leads to labeled lactate acid, suggesting that part of the glutamine oxidation is incomplete. Stoll et al. (30) indicated that this pathway is more important with glucose than with glutamate or glutamine, as observed by the presence of lactate or alanine. Our results are in agreement with these studies; despite the ¹⁴CO₂ production (complete oxidation), a small amount of [¹⁴C]alanine was present, indicating that metabolically significant quantities of glutamine carbons were transformed to pyruvate in enterocytes and that its oxidation was thereby incomplete.

The ATP production derived from glutamine metabolism was essentially derived from ¹⁴CO₂ production, using the stoichiometric ratio of 2 µmol·h⁻¹·cm⁻² CO₂ for 9 µmol·h⁻¹·cm⁻² ATP. This low rate of oxidation would take into account the incomplete glutamine oxidation. The same figure has been used to take into account the probability that a molecule entering the tricarboxylic acid cycle will be oxidized in rat intestinal cells (12). In addition, the glutaminase activity hydrolyzed 0.68 µmol·h⁻¹·cm⁻². We did not use other correcting factors that are probably small and uncertain in these experimental conditions (12). Thus in both control (Fig. 2) and CT (Fig. 3) intestine, the flux of ATP production was ~11 µmol·h⁻¹·cm⁻².

The main objective of the study was to evaluate the effect of CT on energy balance from glutamine metabolism and transport functions. Our results strongly suggest that CT does not alter or minimally alters the energy balance derived from glutamine; in control conditions, the estimated ATP production was 9.8 for 0.46 used for transport, i.e., 95% of the glutamine-derived energy remains available to the mucosa (Fig. 2). Very similar figures were obtained in CT-treated tissues (Fig. 3).

It is likely that the present figures obtained in an isolated piece of intestine mounted in an Ussing chamber are lower than in in vivo conditions. Clearly, the tissue is deprived of its blood supply, and, although oxygenated at pH 7.4 and circulated at 37°C, Ringer solution in the mucosal and serosal compartments does not match the luminal fluid and blood circulation. However, in these well-defined conditions it was possible to study both glutamine metabolism and electrolyte transport in the same piece of tissue. In addition to stimulating Na⁺ absorption, glutamine may also provide energy in CT-treated intestine for other useful functions, including synthesis of key molecules such as glutathione and nucleotides, protein synthesis, epithelial repair, and improvement of barrier function (10, 13, 16, 21, 27).

Finally, we used glutamine as the only energetic substrate in Ringer solution; glutamine was considered as an ingredient for ORS rather than a nutrient provided with food. In addition to stimulating Na⁺ absorption, our results indicate that glutamine given during the rehydration period may have a beneficial effect on intestinal function by providing energy directly to the gut epithelium. These experiments may provide a rationale to test the effect of glutamine in the rehydration period on the absorptive capacity of the intestine during the subsequent feeding period.

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