Distribution of glutaminase and glutamine synthetase activities in the human gastrointestinal tract

L. A. JAMES, P. G. LUNN, S. MIDDLETON* and M. ELIA

MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DQ, U.K., and *Department of Gastroenterology, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.

(Received 4 July/20 October 1997; accepted 24 October 1997)

1. The activities of the two key enzymes involved in glutamine metabolism, glutaminase and glutamine synthetase, were measured in mucosal biopsies taken from different sites throughout the human gastrointestinal tract, from oesophagus to rectum.

2. The specific activity of glutamine synthetase was highest in the stomach (4.5 nmol glutamine formed per minute per mg of protein), but both small and large intestine and the oesophagus had little synthesizing capacity (less than 0.3 nmol of glutamine formed per minute per mg of protein).

3. Glutaminase specific activity was highest in the small intestine (53 nmol glutamate formed per minute per mg of protein by duodenal mucosa), intermediate in the large intestine and lowest in the oesophagus and stomach (less than 13 nmol of glutamate formed per minute per mg of protein).

4. The glutamine concentration in the mucosa was lower in the duodenum than in the colon (0.62 and 0.95 mmol/kg wet weight respectively), but both were much lower than the measured Km values of glutaminases obtained from these sites (3.8 and 4.0 mmol/kg wet weight respectively).

5. The concentration of glutamine in saliva, stomach juice, bile and duodenal juice suggests that very little glutamine passes into the gastrointestinal tract via these secretions.

6. The study provides the most complete information on the distribution of glutamine synthetase and glutaminase along the human gastrointestinal tract, and suggests that (i) both the small and large intestines have a high potential for glutamine metabolism, but little synthesizing capacity, thus both must derive their glutamine from other sources, and (ii) neither the stomach nor the oesophagus have a high glutaminase activity, although the stomach has substantial capacity to synthesize glutamine. The distribution of the enzymes along the gastrointestinal tract may help rationalize the use of glutamine for treating diseases that affect different parts of the gastrointestinal tract.

INTRODUCTION

The arterio-venous catheterization technique offers one of the few ways of studying the metabolism of tissues in vivo, but the inaccessibility of the portal vein in man makes investigation of substrate exchanges across the gut and portal-drained viscera difficult to undertake. As a consequence, there is remarkably little direct information concerning energy and fuel metabolism in the human gastrointestinal (GI) tract. This has led to extrapolation of information from animal studies in vivo, where invasive techniques can be undertaken more readily, and from metabolic studies of intestinal tissue or epithelial cell preparations in vitro [1]. However, all these techniques have important limitations. For example, the net exchange of metabolites across the arterio-portal vasculature does not reflect the metabolism of gut alone, but also that of the portal-drained viscera. The exchange also does not indicate the sites of the GI tract that are most actively involved in metabolism. Furthermore, net measurements of exchange give no indication about the possible bidirectional flux of substrates across the gut and portal-drained viscera, which may be assessed using tracers. Nevertheless the most consistent finding with respect to amino acid exchange across the human gut and portal-drained viscera after an overnight fast [1] is the net uptake of glutamine (40–50 μmol/min, or 8.4–10.5 g/day).

In vitro studies have other important limitations. They frequently fail to take into account [2] the haemodynamic aspects of metabolic control; the perfusing medium may lack important hormones and other regulating signals; only some substrates may be present in the perfusing medium and concentrations may not reflect their luminal and vascular concentrations; the oxygenation and acid–base state may fluctuate during the course of the experiment; and the normal cellular orientation may be lost, especially when isolated cell preparations are used.

Key words: gastrointestinal tract, glutamine, gut.
Abbreviation: GI, gastrointestinal.
Correspondence: Dr M. Elia.
Clinical interest in glutamine has arisen in part from its multiple metabolic functions and its GI effects: it induces intestinal hyperplasia [3–5], and improves gut-barrier function [6] and blood flow to the gut [7]; it is essential for the synthesis of nucleic acids and amino sugars constituting part of the mucus barrier; it is a major energy source for the mucosa [1] and for gut-associated lymphocytes [8, 9] and it is co-transported with sodium across the mucosa [10, 11], possibly preventing diarrhoea and dehydration. Interest has also arisen from remarkable animal studies which have demonstrated that glutamine supplementation reduces morbidity and mortality associated with enterocolitis [12, 13] due to cytotoxic drugs or radiotherapy. Beneficial effects have also been reported in pigs suffering from GI rotavirus infection [14]. As a result a variety of therapeutic studies with pharmacological doses of glutamine have been undertaken in man in a wide range of conditions affecting different parts of the GI tract [15]. Among these is mucositis of the upper and lower GI tract, which can result from the use of aggressive chemotherapy or radiotherapy to treat malignant conditions [15–18]. Glutamine has also been used, with reported success, to treat peptic ulcers [19, 20] and inflammatory conditions of the intestine, e.g. pouchitis [21]. Despite these clinical applications, information about glutamine metabolism in different segments of the human GI tract is fragmentary, or absent. For example, as far as we are aware, there is no information about glutamine metabolism or enzyme activities in the mucosa of the upper GI tract, which has stratified squamous epithelium. Therefore therapeutic studies involving the use of glutamine to treat inflammatory conditions of the mouth and oesophagus have taken place without this basic biochemical information.

Another way of assessing the metabolism of glutamine in the human GI tract is to measure the activities of key enzymes. Although this may not necessarily reflect the actual rate of exchange of glutamine in vivo, it can provide important information about the capacity of different parts of the gut to synthesize and utilize glutamine. However, there is a striking lack of information about the characteristics and distribution along the human GI tract of key enzymes involved in glutamine metabolism: glutamine synthetase (EC 6.3.1.2) and glutaminase (EC 3.5.1.2) [22, 23].

This study aimed to assess systematically the distribution of glutaminase and glutamine synthetase along the mucosa of the GI tract, to measure the kinetic characteristics of glutaminase and to relate the findings to the mucosal concentrations of glutamine and glutamate. It also aimed to assess whether the GI tract is exposed to substantial amounts of glutamine secreted into the lumen of the gut. Finally, the study aimed to obtain insights into the variable therapeutic success of glutamine administration to treat various conditions affecting the GI tract.

METHODS

The study described was approved by the Local Research Ethics Committee, Addenbrooke's Hospital NHS Trust, and full informed consent was obtained from all subjects before taking samples.

Small biopsy samples with wet weight ranging from 2.0 to 15.3 mg were obtained from the GI tract of subjects aged 35–65 years undergoing endoscopic examination for dyspepsia, or colonic examination for recurrence of colonic polyps. In six subjects mucosa was obtained from the oesophagus, the upper and lower stomach, and the duodenum. In a further six subjects biopsies were obtained from six sites in the large intestine, the caecum, ascending, transverse descending and sigmoid colon and rectum and also from the ileum. Specimens for analysis were taken only from subjects who had no apparent structural GI abnormalities. None of the subjects was taking steroids, non-steroidal anti-inflammatory drugs, H2 receptor antagonists or calcium-channel blockers such as omeprazole. Histological examination showed the samples were normal.

Saliva samples were obtained from fasting volunteers. Gastric juice was aspirated from 20 adult subjects undergoing endoscopic examination but with no apparent GI abnormalities. Bile and duodenal juice samples were obtained from patients undergoing endoscopic retrograde cholangio-pancreatography. Bile samples were collected by cannulation of the bile duct and duodenal juice directly from the duodenum before administration of the radio-opaque dye.

Enzyme assays

Tissue biopsy samples were immediately weighed and homogenized (15 000 r.p.m. for 20–30 s) in 700 µl of ice-cold buffer (50 mmol/l Tris containing 2 mmol/l EDTA, pH 7.9) using an Omni 1000 homogenizer (Omni International, Waterbury, CT, U.S.A.).

The activity of glutaminase was measured using a radiochemical technique based on the method of Fox et al. [12]. An aliquot of each homogenate was further diluted 10-fold with extraction buffer and 50 µl of the suspension was incubated with 0.05 µCi of L-[14C]glutamine (80 mCi/mmol) plus 4 mmol/l unlabelled glutamine, 1.0 mmol/l potassium cyanide, 8.0 mmol/l imidazole and 150 mmol/l potassium phosphate buffer at pH 8.2, in a total volume of 0.25 ml. After incubation for 30 min at 37°C, the reaction was terminated by addition of 1 ml of ice-cold 20 mmol/l imidazole–HCl buffer, pH 7.5. Samples were centrifuged at 2000 g for 5 min and 1 ml of supernatant was loaded on to a 4 ml column of AG-1X8 anion exchange resin (200–400 mesh, chloride form) (Bio-Rad) which had been equilibrated with 20 mmol/l imidazole–HCl buffer, pH 7.5. The column was washed sequentially with 7 ml of imidazole buffer, then 1 ml of 0.1 mol/l HCl.
Glutamine metabolism in human gastrointestinal tract

Mass 620 g; length 500 cm; diameter of lumen 3 cm; thickness of mucosal layer minus villi 0.025 cm; hence mass of mucosa minus villi = 118 g. Volume of single villus 9.3 x 10^{-6} cm^3; number of villi 7500/cm^2; hence total mass of villi = 230 g and therefore total mucosal mass = 348 g (56.1% of total mass).

Total duodenal mass is 60 g, hence mucosal mass = 34 g; total jejunal mass is 265 g, hence mucosal mass = 149 g; total mass of ileum is 295 g, hence mucosal mass = 166 g.

Large intestine

Mass 365 g; length 160 cm; diameter of lumen 4 cm; thickness of mucosa 0.06 cm; hence mucosal mass = 121 g (33% of total mass).

Total mass of caecum is 16 g, hence mucosal mass = 5.3 g; total mass of ascending colon is 41 g, hence mucosal mass = 13.6 g; total mass of transverse colon is 114 g, hence mucosal mass = 37.7 g; total mass of descending colon is 69 g, hence mucosal mass = 22.6 g; total mass of sigmoid colon is 91 g, hence mucosal mass = 30.1 g; total mass of rectum = 34 g, hence mucosal mass = 11.3 g.

Full details of the calculations are given in James [27].

Statistical significance was assessed by Student's t-test or analysis of variance as appropriate, using SPSS (Statistical Package for Social Scientists) software, version 3.01. Significance was assumed if P < 0.05.

RESULTS

Glutamine synthetase

The specific activity of this enzyme (Fig. 1) was far greater in the mucosa of the upper stomach (4.43 nmol glutamine min^{-1} mg^{-1} protein) than at any other site in the GI tract (0.03-0.38 nmol glutamine min^{-1} mg^{-1} protein) (P < 0.001 by analysis of variance). Activity in the lower part of the stomach was higher than that in the small intestine (P < 0.05), but there were no significant differences between other sections of the GI tract. When expressed as total glutamine synthesized per section of the GI tract using the mucosal mass data (Fig. 2), the stomach accounted for 55% of total activity, the small and large intestines for 32% and 11% respectively, while the oesophagus contained less than 2%. Total mucosal glutamine synthetase activity in the

...
GI tract was estimated at 16.2 μmol of glutamine formed per minute.

**Glutaminase**

The highest specific activity of glutaminase was observed in the small intestine, reaching 53 nmol glutamate min⁻¹ mg⁻¹ protein in the duodenum (Fig. 3). Lower ($P < 0.05$ by analysis of variance), but still substantial activity (20–32 nmol glutamate min⁻¹ mg⁻¹ protein) was found in the large intestine and activity of this enzyme was at its lowest in the stomach and oesophagus, (6–16 nmol glutamate min⁻¹ mg⁻¹ protein) ($P < 0.001$ and $P < 0.05$ compared with small and large intestine respectively). Estimates of total activity (Fig. 4) showed that the small intestine contained 84% of total glutaminase activity in the GI tract. The large intestine accounted for 15% of total activity but the oesophagus and stomach combined accounted for only 1.4%. The total mucosal activity of the GI tract was estimated as 2643 μmol of glutamate formed per minute.

**Kinetic parameters of glutaminase**

Maximum velocity ($V_{\text{max}}$, the rate of reaction with saturating concentration of substrate) and the Michaelis constant ($K_{\text{m}}$, the concentration of substrate at half maximum velocity) were determined in mucosal samples from the duodenum and descending colon (Table 1), using methods described by James [27]. Due to the larger amount of enzyme present, $V_{\text{max}}$ was significantly higher in the duodenum than the descending colon ($P < 0.01$), but the
Table 1. Kinetic parameters of phosphate-dependent glutaminase from the small and large intestinal mucosa of humans. Values are means ± SD, n = number of observations. Results of the duodenum and descending colon are significantly different. **P<0.01 (Student’s t-test).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>( V_{max} ) (nmol glutamate min⁻¹ mg⁻¹ protein)</th>
<th>( K_m ) (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>5</td>
<td>101.7 ± 26.4</td>
<td>3.77 ± 0.51</td>
</tr>
<tr>
<td>Descending colon</td>
<td>5</td>
<td>44.4 ± 8.8**</td>
<td>4.00 ± 0.84</td>
</tr>
</tbody>
</table>

\( K_m \) values for the two tissues were very similar (3.77 and 4.00 respectively).

### Mucosal concentrations of glutamine and glutamate

Glutamine and glutamate concentrations in the duodenum and descending colon are shown in Table 2. Both tissues contained similarly large quantities of glutamate, but much lower amounts of glutamine. Glutamine concentration was significantly lower in the duodenum compared with the descending colon (P<0.01).

### GI juices

As the results in Table 3 demonstrate, concentrations of both glutamine and glutamate were very low in all the GI juices tested. Even the highest glutamine concentration found, i.e. 70.6 μmol/l in stomach juice, was only about one-tenth of normal blood concentration. Neither glutamine nor glutamate were detected in bile although recovery of added glutamine or glutamate to this secretion was close to 100%.

Table 3 also gives estimates of the total daily amount of glutamine and glutamate entering the GI tract via these juices. The total amount of glutamine secreted into the GI tract was estimated to be 207 μmol/day (approximately 30 mg/day), which is clearly insignificant when compared with a normal daily dietary intake of about 5 g.

### DISCUSSION

This study provides the first comprehensive examination of the distribution of the two key glutamine-metabolizing enzymes (glutamine synthetase and glutaminase) throughout the GI tract of humans. The enzymic technique employed provides insights into the capacity of specific sites to synthesize and catabolise glutamine.

In comparison with the upper stomach glutamine synthetase activity was low in all regions of the GI tract, suggesting that the human gut depends almost entirely on vascular glutamine derived from other tissues, and luminal glutamine derived from the diet or GI secretions. The measured concentrations of glutamine in the GI secretions (bile, saliva, gastric and duodenal juice) suggest that such secretions contribute little to the luminal supply of glutamine. Dietary glutamine is considered to be much more important (up to about 5 g/day), and this in turn is considered less important than the vascular supply of glutamine [1]. The activity of glutamine synthetase in the stomach, which represents more than half of the total mucosal activity of the GI tract, was not associated with high glutamine concentrations in gastric juice.

Using the methods described, the total activity of glutaminase in the mucosa of the GI tract (more than 80% in the small intestine) is approximately

### Table 2. Concentrations of free glutamine and glutamate in the duodenum and descending colon of overnight fasted humans. Values are means ± SD, with number of separate experiments given in parentheses. Results of the duodenum and descending colon are significantly different. **P<0.01 (Student’s t-test).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine (mmol/kg wet weight)</th>
<th>Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>0.62 ± 0.22 (5)</td>
<td>3.96 ± 0.46 (5)</td>
</tr>
<tr>
<td>Descending col.</td>
<td>0.95 ± 0.21** (5)</td>
<td>3.81 ± 0.61 (5)</td>
</tr>
</tbody>
</table>

### Table 3. Glutamine and glutamate concentrations and estimated daily secretion into gastrointestinal tract juices. *Volumes of juices secreted per day were assumed to be saliva 1.5 litres; gastric juices 2.5 litres; bile 0.6 litre; and duodenal juice (excluding pancreatic juice) 1.0 litre [32]; and the unstimulated fasting concentrations are assumed to be similar to concentrations in stimulated secretions.

<table>
<thead>
<tr>
<th></th>
<th>Glutamine</th>
<th>Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. (μmol/l)</td>
<td>Estimated daily secretion* (μmol/day)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Saliva</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td>Gastric juice</td>
<td>20</td>
<td>70.6</td>
</tr>
<tr>
<td>Bile</td>
<td>7</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Duodenal juice</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>207.1</td>
<td></td>
</tr>
</tbody>
</table>
2600 μmol of glutamine hydrolysed per minute, some 40 times greater than the estimated net splanchnic uptake of glutamine, as determined by arterio-venous exchange. This discrepancy can at least partly be attributed to the difference between the in vivo and in vitro glutamine concentrations. The observed $K_m$ values of glutaminases from the duodenum and descending colon were similar (3.77 and 4.00 mmol/l respectively). Although lower than those reported by Sarantos et al. [22] in studies of isolated colonic cells, the values are substantially greater than the mucosal concentrations of glutamine (0.62 and 0.95 nmol/kg wet tissue).

Glutamate concentrations were high in both the duodenum and descending colon (3.96 and 3.81 mmol/kg wet weight respectively), but glutamine concentration was lower in the duodenum compared with the descending colon (0.62 and 0.95 mmol/kg wet weight respectively). The lower glutamine concentration in the duodenum is consistent with a higher glutaminase activity and a higher rate of glutamine utilization in the duodenum compared with the descending colon.

The lowest glutaminase activity was found in the mucosa of the upper GI tract (especially mucosa with stratified squamous epithelium). This is consistent with lack of effect of glutamine in treating mucositis of the upper GI tract [16, 17]. The highest activity of glutaminase was in the small intestine, and this is consistent with the putative benefits of glutamine in treating inflammatory conditions of the small intestine [15, 21]. The activity of glutaminase in the large intestine is intermediate (and lower than that found in colonocytes) [22], but clinical studies on the effects of glutamine in inflammatory conditions of the large bowel are lacking. In experimentally induced colitis in the rat [28] rectal glutamine administration failed to produce benefit. However, caution should be exercised in extrapolating results to the human, partly because the animal model of colitis may not reflect the human condition, and partly because the enzyme activities in the rat are different from those in the human [27, 29, 30]. For example, the ratio of colonic glutaminase activity (percentage of total GI tract mucosal activity) to colonic glutamine synthetase activity is 4-fold greater in the human than in the rat. Furthermore, there is virtually no information about enzyme activities in diseases of the GI tract.

Another enzymic difference between rat and human is that the only part of the rat GI tract that has substantial mucosal activity of glutamine synthetase is the lower stomach, whereas in man it is the upper stomach. Since in man the upper stomach is less frequently affected by peptic ulceration, it is reasonable to hypothesize that the production of glutamine in this part of the stomach has a protective role, especially since glutamine has been reported to successfully prevent or treat peptic ulcers in both humans and rats [19, 20]. However, the rat also tends to develop ulcers in the lower stomach (at least in response to aspirin). Therefore the significance of the species difference in the distribution of glutamine synthetase is uncertain.

Current is there is insufficient information regarding glutamine synthetase and glutaminase activities in other organs of the body in humans. It is therefore difficult to estimate the relative contribution of the mucosa of the GI tract in terms of whole-body glutaminase activity. However, arterio-venous uptake data suggest that the gut and portal-drained viscera account for more than half the splanchnic uptake (15 g/day) [31] after an overnight fast. Furthermore, this uptake by the gut is far greater than the net daily uptake of glutamine by other organs such as the brain and kidney [31].

In summary, this is the first comprehensive study which maps out the distribution of glutaminase and glutamine synthetase in the human gut. It also provides the first report of enzyme activities in the oesophagus and at various other sites of the GI tract, including the caecum. In a similar manner to the rat, these data reveal that there is a differential distribution of glutaminase and glutamine synthetase along the length of the GI tract in humans. Both the small and large intestine have a high capacity for glutamine metabolism, but very limited capacity for synthesis, and thus both must be expected to derive their glutamine from other sources. On the other hand, mucosa of the upper stomach has potential for synthesizing glutamine. Such information may help rationalize the use of glutamine for treating diseases that affect different parts of the GI tract. For example, the results suggest that glutamine supplementation is unlikely to be helpful in treating mucositis of the upper GI tract [16, 17] but is more likely to be beneficial in mucositis of the small intestine [15, 18]. It is unclear whether inflammatory conditions of the colon (which showed intermediate glutaminase activity in normal tissue) would respond to glutamine administration. However, the effect of disease on glutaminase activity in these tissues remains to be established.

ACKNOWLEDGMENT

We wish to thank Dr G. Neale for his help with the study.

REFERENCES

10. Fox
12. Jebb
13. Rhoads JM
16. Jebb
5. Gianotti