Glutamine and the Bowel

Peter J. Reeds and Douglas G. Burrin

ABSTRACT Since the pioneering work of Windmueller and Spaeth, the importance of glutamine to the support of intestinal mucosal metabolic function has become generally accepted. Nevertheless, the mechanisms underlying this role still remain obscure. This paper explores a number of questions: 1) Is glutamine essential for intestinal function? 2) To what extent does this relate to its intermediary metabolism? 3) What is the importance of glutamine as a biosynthetic precursor? 4) Is glutamine supplementation of the nutrient mixture presented to patients of any metabolic or clinical benefit? As a result of this exploratory exercise, the following general conclusions were reached: 1) Much suggestive biochemical and physiologic evidence exists that implies that glutamine, especially systemic glutamine, supports the function of the intestinal mucosal system. 2) Despite the extensive metabolic role of this amino acid by the intestinal tissues, most evidence suggests that if glutamine does play a physiologic role in the bowel, it is not compellingly related to its intermediary metabolism. 3) There is, on the other hand, evidence that the mucosal cells not only utilize extracellular glutamine but synthesize the amino acid. Given that inhibition of glutamine synthesis inhibits both proliferation and differentiation of mucosal cell cultures, this suggests some more subtle regulatory role. This notion is supported by the demonstration that glutamine will activate a number of genes associated with cell cycle progression in the mucosa. 4) Despite the accumulated evidence, the mechanisms underlying glutamine’s function and the question whether glutamine supplementation uniformly benefits mucosal health remain equivocal at best.

KEY WORDS: glutamine metabolism, intestine, glutamate

The traditional view of the intestine has focused on its function as an organ of digestion, nutrient absorption and fermentation. However, it has become very clear that the intestine is a complex, multicellular organ that performs a number of critical physiologic functions that are separate from its role in nutrient assimilation. Thus, the intestinal mucosa contains secretory, immune and neuroendocrine cells in addition to the absorptive enterocytes. As such, the intestinal tissues are involved in immune surveillance and in generating endocrine responses to the lumenal environment. These regulatory roles are supported by an intrinsic intestinal neural system (Kudsk 2000) that is separate from, but functionally related to the central neural pathways. Thus, the intestine is one partner in a central-peripheral system that senses both the antigenic and the nutritional environment and thereby modifies the host response.

The host pays a metabolic price for these critical intestinal functions. The portal drained viscera (the stomach, intestine, pancreas and spleen) are among the most metabolically active tissues in the body. For example, although these tissues collectively never account for >6% of body weight, they can be responsible for up to 50% of the whole-body turnover of some essential amino acids (Stoll et al. 1998, Yu et al. 1992 and 1995) and between 10 and 20% of whole-body energy expenditure (van Goudoever et al. 2000). For these reasons alone, the examination of the substrates that are used by the intestinal tissues and the potential for nutrient regulation of the intestine’s multiple functions is a subject worthy of intensive study.

In 1974, Windmueller and Spaeth published the first of a series of highly influential papers (Windmueller and Spaeth 1974, 1975, 1976 and 1980) in which they demonstrated that amino acids, especially nonessential amino acids have an important metabolic role in the intestine. However, from the perspective of the present discussion, they made the crucial observation that the intestine removes as much as 25% of the systemic flux of glutamine. Their measurements of intestinal glutamine metabolism also showed that glutamine metabolism could not only contribute a nutritionally important portion of intestinal energy generation, but that the amino acid was the precursor for a number of important metabolic pathways, especially those leading to the synthesis of ornithine, citrulline,

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requirement for mucosal health. For example, even though glutamine amide nitrogen is an obligatory substitute for glutamine in the pathways that involve the oxidation of glutamine? Second, to what extent can glutamate necessary oxidation of the glutamate released from deamination oxidation in the mucosa simply a reflection of the two further questions: first, is the high fractional rate of glutamate and free ammonia. This latter factor, in its turn, leads to the skeleton of glutamine involves its initial conversion to glutamine intermediary metabolism in the intestinal mucosa. On the other hand, the utilization of the carbon chain of glutamine involves its initial conversion to glutamine. The amide group of glutamine enter pathways. In the first, the amide nitrogen of glutamine is utilized in support of purine, pyrimidine and amino sugar synthesis. In the second, the carbon chain and α-amino group of glutamine enter pathways that lead to the synthesis of other amino acids, notably proline, ornithine and arginine (Wu 1998). This biochemical division of glutamine metabolism also reflects an intracellular compartmentalization, in as much as purine, pyrimidine and amino sugar synthesis are cytoplasmic activities, whereas the metabolism of the carbon skeleton of glutamine is initiated by its deamination by mitochondrial phosphate-dependent glutaminase (Curthoys and Watford 1995).

This metabolic division is also important with regard to the question whether glutamine is necessary for mucosal metabolism. On the one hand, the fact that the amide group of glutamine is important specifically for nucleic and mucin glycosylation implies strongly that the presence of glutamine is obligatory for the maintenance of proliferative and secretory activity. On the other hand, the utilization of the carbon skeleton of glutamine involves its initial conversion to glutamate and free ammonia. This latter factor, in its turn, leads to two further questions: first, is the high fractional rate of glutamine oxidation in the mucosa simply a reflection of the necessary oxidation of the glutamate released from the deamination of glutamine? Second, to what extent can glutamate substitute for glutamine in the pathways that involve the utilization of the carbon and amino nitrogen moieties?

There are, as we can ascertain, no in vivo data to identify directly whether glutamine amide nitrogen is an obligatory requirement for mucosal health. For example, even though glutamine starvation of isolated intestinal mucosal cell lines does substantially inhibit their proliferation, there is evidence to suggest that in regard to the support of small intestinal mucosal mass (Horvath et al. 1996) and protein synthesis (Hasebe et al. 1999), glutamate is as effective as glutamine. Moreover, those pathways of mucosal intermediary metabolism that utilize the carbon skeleton of glutamine can apparently utilize glutamate equally well. In fact it seems that both in vitro (Wu 1997, Wu et al. 2000, Wu and Reeds, unpublished data; Fig. 1) and in vivo (Brunton et al. 1999), proline is a more effective substrate for arginine, ornithine and polyamine synthesis than either glutamine or glutamate. The same apparently applies to the effectiveness of glutamate as a precursor for mucosal glutathione synthesis (Reeds et al. 1997). Moreover, in humans, the first-pass fractional extraction of dietary glutamate is greater than that of enteral glutamine (Matthews et al. 1993), suggesting that the metabolism of glutamine taken up from the mesenteric artery is more extensive than that of glutamine absorbed from the intestinal lumen. Limited evidence in pigs suggests much the same conclusion (Stoll et al. 1999), and in these studies, substantially more visceral CO₂ was generated from glutamate and glucose metabolism than from glutamine (Fig. 2). At this stage then, we would conclude that from a strictly metabolic perspective, glutamate and glutamine are interchangeable as important substrates for the mucosal cellular system.

**New roles for glutamine in the gut**

Despite the conclusion that we reached in the previous section, there is increasing evidence that favors not only a specific role for glutamine but also the idea that this role may not be strictly metabolic. The first evidence is the crucially important observation that extracellular glutamine is not only removed from the arterial circulation by the intestinal tissues, but that the mucosal cells in both the crypt and villous compartments are simultaneously synthesizing glutamine. Thus, Neu and his colleagues (Shenoy et al. 1996) published immunocytochemical evidence for the presence of glutamine synthase in the mucosa, and James et al. (1998) demonstrated the presence of the enzyme by direct biochemical measurement.

**TABLE 1**

<table>
<thead>
<tr>
<th>Amido-N end products</th>
<th>Intermediary metabolic products</th>
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<tbody>
<tr>
<td>Purine</td>
<td>Ornithine</td>
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<tr>
<td>Pyrimidine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Amino sugars</td>
<td>Proline</td>
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<tr>
<td></td>
<td>Polyamines</td>
</tr>
<tr>
<td></td>
<td>Ammonia¹</td>
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<tr>
<td></td>
<td>Alanine¹</td>
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¹ Catabolic end products released to the liver and used in urea synthesis.

proline and arginine [see Wu (1998) for further discussion]. Although they studied the metabolism of other amino acids, especially glutamate and aspartate (Windmueller and Spaeth 1980), their observations of glutamine metabolism have had a substantial influence on a number of aspects of clinical nutrition and have spawned a substantial literature on the role of glutamine in the bowel (Hall et al. 1996, Smith and Wilmore 1990).

In this paper we wish to survey aspects of this literature and discuss a number of general questions as follows: 1) Is glutamine essential for intestinal function? 2) To what extent does this relate to its metabolic role? 3) What is the importance of glutamine as a biosynthetic precursor? 4) Is glutamine supplementation of the nutrient mixture presented to patients of any metabolic or clinical benefit?

**Glutamine, glutamate and mucosal metabolism**

From a metabolic perspective, glutamine metabolism essentially follows two functional pathways (Table 1). In the first, the amide nitrogen of glutamine is utilized in support of purine, pyrimidine and amino sugar synthesis. In the second, the carbon chain and α-amino group of glutamine enter pathways that lead to the synthesis of other amino acids, notably proline, ornithine and arginine (Wu 1998). This biochemical division of glutamine metabolism also reflects an intracellular compartmentalization, in as much as purine, pyrimidine and amino sugar synthesis are cytoplasmic activities, whereas the metabolism of the carbon skeleton of glutamine is initiated by its deamination by mitochondrial phosphate-dependent glutaminase (Curthoys and Watford 1995).

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**FIGURE 1** Metabolic interrelationships among amino acids in isolated enterocytes. Porcine enterocytes were isolated and incubated in a complete medium containing [U-¹³C]glutamate, glutamine, glucose or proline. The data are the proportion of the intracellular flux of their potential products. (Unpublished data of G. Wu and P. J. Reeds.)
Does glutamine make a difference?

When the evidence that suggests a specific role for glutamine in the bowel is taken with the alterations in interorgan flow of glutamine (Soubra and Austgen 1990) and intestinal protective function (Stechmiller et al. 1997) that accompany stress in general and intestinal disease in particular [reviewed by Elia and Lunn (1997), Fürst (2000) and Sacks (1999)].

We think that it is fair to say that not only does the literature report confusing and variable success but also serves to emphasize the following. First, it is crucial to identify the desired end point of glutamine supplementation. Second, it is equally important to define the nature of the stress or disease that it is hoped will be ameliorated with glutamine. Third, it is more than likely that the route of glutamine supplementation (parenteral or enteral) influences the response and, finally, other aspects of the nutritional support of the patient are of extreme importance.

Among these considerations, we would argue that the question of end point is the most crucial, and the effects of glutamine on nitrogen metabolism provide a good example of this. Thus, in the eight studies of parenteral glutamine supplementation identified by Sacks (1999) in which measurements of nitrogen balance were made, there were uniform increases in circulating glutamine concentrations and improved nitrogen balance. On the other hand, clinically demonstrable benefit was not a uniform finding. Conversely, in 18 studies of enteral glutamine supplementation (Fürst 2000), there are no reports of significantly improved nitrogen balance, but a number of reports of improved morbidity. Even so, the benefits have not been uniform (Elia and Lunn 1997). By and large, we would conclude that effects of glutamine supplements on mucosal mass, even in animal models, have been equivocal at best.

Despite this rather negative conclusion, the emergence of new roles for glutamine suggests other areas in which glutamine supplements may prove to be of benefit. In this regard, one promising area is the putative role of glutamine in amino acid synthesis. This role has two potential implications. First, by influencing the synthesis of components of the extracellular matrix, glutamine may be one factor in the maintenance of mucosal structure, especially the maintenance of tight junctions (Panigrahi et al. 1997). Second, by being a potential precursor for N-acetylglucosamine and N-acetylgalactosamine synthesis, glutamine could play a critical role in intestinal mucin synthesis and hence in the maintenance of the passive barrier to bacterial ingress (Khan et al. 1999). In this regard, one of the most interesting recent papers concerned with enteral glutamine supplementation of the diets of low-birthweight infants (Neu et al. 1997), the supplement had no effect on either circulating glutamine concentrations or infant growth but was associated with changes in immune cell sub-type distribution that were compatible with the idea that the supplement had lowered the overall immune challenge presented to the infants. Whether this reflected the maintenance of tight junctions and mucin synthesis (Khan et al. 1999) or whether it reflected interactions with locally generated cytokines (Kudsk et al. 2000) is not known at this time.

CONCLUSIONS

We have sought to make three main points. First, there is much suggestive biochemical and physiologic evidence that glutamine, especially systemic glutamine, supports the function of the intestinal mucosal system. Second, despite the extensive metabolism of this amino acid by the intestinal tissues, most evidence suggests that it glutamine does play a physiologic role in the bowel, it is not compellingly related to its intermediary metabolism. In fact, glutamate and proline, especially derived from the diet, can readily substitute for many of the metabolic roles of glutamine, including energy

FIGURE 2

Contributors to intestinal CO2 production in well-nourished piglets. Pigs (8 kg) were catheterized in the carotid artery and the jugular and hepatic portal veins. Pigs were fed by intragastric diet infusion and were given enteral [U-13C] glutamate or [1-13C] leucine or intravenous [U-13C] glucose, glutamine or [1-13C] leucine. Portal blood flow and arterial and portal 13CO2 concentrations and isotopic enrichments were measured. Values are the proportion of total visceral CO2 production attributable to the respective substrates. [Data from Stoll et al. (1999) and S. Van der Schoor unpublished.]
generation and amino acid synthesis. Third, there is, on the other hand, evidence that the mucosal cells not only utilize extracellular glutamine but also synthesize the amino acid. Given that inhibition of glutamine synthesis inhibits both proliferation and differentiation of mucosal cell cultures, it seems that glutamine may be performing some more subtle regulatory role. This notion is supported by the demonstration that glutamine will activate a number of genes associated with cell cycle progression in the mucosal cells. However, despite the accumulated evidence, both the mechanism underlying glutamine’s function and whether glutamine supplementation uniformly benefits mucosal health remain, at best, equivocal.

LITERATURE CITED


