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Glutamine metabolism in rat small intestine: synthesis of three-carbon products in isolated enterocytes

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Abstract

Glutamine is a major respiratory fuel for enterocytes but the extent of glutamine decarboxylation in these cells is not certain. The metabolism of differentially labeled L-[^{14}C]glutamine was studied in enterocytes isolated from fed rats. The results indicate that glutamine undergoes two decarboxylations and yields a three carbon end product. The first decarboxylation is presumably at α-ketoglutarate dehydrogenase but the identity of the second reaction is not clear. The addition of 3-mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase, was without effect on either the rate of glutamine metabolism or the extent of decarboxylation. Labeled glutamine carbon was recovered in three carbon products primarily as alanine with lesser amounts as lactate. The addition of glucose to the incubation medium did not change the rate of glutamine metabolism, or decarboxylation, but lactate became the major labeled three carbon end product. The results show that the fate, alanine or lactate, of glutamine derived pyruvate in enterocytes depends on the relative rate of flux through pyruvate and indicates that one cytosolic pool of pyruvate exists in these cells. The limited oxidation of glutamine in enterocytes ensures that the gluconeogenic potential of glutamine is conserved within the body.

Key words: Glutamine; Enterocyte; Intestine; (Rat)

1. Introduction

Glutamine is recognized as a major respiratory substrate for many mammalian cell types [1–3], including enterocytes, cells in culture, reticulocytes and cells of the immune system. In the body, under normal physiological conditions, enterocytes of the small intestine are quantitatively the most significant in terms of glutamine utilization [3]. Neptune [4] was first to report high rates of glutamine oxidation in intestinal preparations. This was confirmed by the elegant studies of Windmueller and Spaeth [5–8] who reported that glutamine was the major respiratory fuel of rat small intestine and that glutamine oxidation could account for $> 30\%$ of the CO_2 produced by this organ. Other products of intestinal glutamine metabolism include alanine, lactate, citrulline, proline and ammonia. The initial studies of Windmueller and Spaeth [5–8] showed that, although considerable glutamine nitrogen was recovered as alanine, less than 3% of glutamine carbon was converted to alanine. Windmueller and Spaeth also showed that complete oxidation of glutamine in the intestine was unlikely since approx. 55–64% of the glutamine carbon was recovered as CO_2, indicating that a significant proportion of glutamine is metabolized to additional products. Such results are indicative of three decarboxylations per glutamine molecule, although there is no evidence of a two carbon end product. A number of reviews and publications over the past 10–15 yr indicate considerable confusion as the extent of glutamine decarboxylation in the small intestine [3,9–11].

From studies, in this laboratory, of glutamine metabolism during uncontrolled diabetes [12], and also of the role of intestinal phosphoenolpyruvate carboxykinase (proposed to play a role in the formation of pyruvate from TCA cycle intermediates) [13], it became apparent that there was little or no evidence for the complete oxidation of glutamine carbon by rat enterocytes. This has important physiological consequences,

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since complete oxidation of glutamine would represent the loss of gluconeogenic precursors from the body whereas partial oxidation to a three carbon intermediate would preserve gluconeogenic potential. The work described in this paper was designed to address the question of the fate of glutamine carbon in rat enterocytes by comparing the rate of $^{14}CO_2$ production from 1, 5 or U labeled glutamine. The results indicate that glutamine always undergoes two decarboxylations and that the labeled three carbon end-product is either alanine or lactate depending on the rate of flux through the pyruvate pool.

2. Materials and methods

2.1. Rats

Male Sprague-Dawley rats (200–300 g) obtained from Blue-Spruce (Alamont, NY, USA) were maintained on a standard diet (Purina) and water ad libitum. Animal rooms were illuminated from 7 am to 7 pm and all experiments were begun between 8–10 am.

2.2. Isolated enterocytes and metabolite assays

Enterocytes were isolated by means of EDTA and incubated as previously described [14]. Cells (8–10 mg dry wt) were incubated in a final volume of 4.0 ml of Krebs-Henseleit bicarbonate buffered saline for up to 30 min. at 37°C. Glutamine labeled in either the 1,5 or U positions was present in the initial incubation media at similar specific activities (approx. 25 000 dpm/μmol glutamine). Incubations were stopped by the addition of 1.0 ml 10% perchloric acid and CO_2 was collected for liquid scintillation counting in 0.2 ml KOH in a disposable center-well (Kontes, Vineland, NJ, USA). Cell viability was assessed by leakage of lactate dehydrogenase during the course of incubation and in all cases was $< 6\%$. Metabolites; glutamine, glutamate, alanine, aspartate, lactate, pyruvate, malate, α-ketoglutarate, and ammonia, were determined by enzymatic methods as previously described [12,14]. Glutamine carbon oxidized to CO_2 was calculated after correction for non-specific CO_2 production estimated in a control flask without cells. Radioactivity in organic and amino acids was determined by two-dimensional thin layer chromatography. Initial trials showed no detectable label in malate, α-ketoglutarate, citrate or fumarate. The method of Myers and Huang [15] was used which separates amino acids from organic acids into two separate sections of the plate, the upper section ($Rf > 50$ in Ether:formic acid: water, 7:2:1) giving lactate and pyruvate with all amino acids remaining on the bottom half of the plate. The second solvent (phenol:water:formic acid, 75:25:1) effectively separates the individual amino and organic acids. The amino acid section of the plate was sprayed with ninhridin to detect aminoacids and the remainder with bromocresol blue to detect organic acids. Spots were scraped from the plates and radioactivity determined by liquid scintillation counting.

2.3. Chemicals

L-[U-^{14}C]glutamine and L-[1-^{14}C]glutamate were purchased from New England Nuclear. [5-^{14}C α-ketoglutarate was from Amersham. L-[1-^{14}C]-glutamine was synthesized according to the procedure of Squires and Brosnan. L-[5-^{14}C]-glutamate was synthesized from [5-^{14}C]-α-ketoglutarate (20 μCi), NADH (0.5 mg), glutamate dehydrogenase in NH_4SO_4, in 1 ml 0.2 M potassium phosphate buffer, pH 7.4, for 30 min at 37°C followed by conversion to L-[5-^{14}C]-glutamine using

Table 1
Metabolism of glutamine in isolated enterocytes Enterocytes were incubated in the presence or absence of labeled glutamine as described in Section 2. Results are expressed as μmol metabolite removed (-) or produced (+) per min per g dry wt. and are mean ± SEM for 4 enterocyte preparations. Abbreviations used; β-OH, β-hydroxybutyrate; AcAc, acetoacetate; 3-MCPA, 3-mercaptopicolinate.

	Metabolite (μmol/min/g dry wt)						
Incubation	Glutamine	Glutamate	Alanine	Aspartate	Ammonia	Lactate	Pyruvate
Glutamine (2 mM)	-9.37	+3.27	+2.97	+0.55	+9.35	+0.78	+0.26
	±0.83	±0.23	±0.39	±0.39	±0.90	±0.08	±0.04
Glutamine (2 mM)	-9.48	+2.50	+5.00	+0.29	+7.24	+12.7	+1.68
+ Glucose (10 mM)	±0.68	±0.32	±0.36	±0.12	±0.16	±1.20	±0.08
Glutamine (2 mM)	-9.07	+3.20	+2.69	+0.55	+9.05	+0.82	+0.21
+βOH (3 mM)	±0.34	±0.23	±0.46	±0.13	±1.03	±0.02	±0.04
+ AcAc (1 mM)							
Glutamine (2 mM)	-8.83	+3.14	+2.74	+0.86	+7.73	+0.88	+0.46
+3-MCPA (1 mM)	±0.42	±0.20	±0.33	±0.13	±0.89	±0.14	±0.05
Glucose (10 mM)	+0.12	+0.15	+0.70	+0.06	+0.71	+15.8	+1.60
	±0.07	±0.06	±0.15	±0.04	±0.27	±1.47	±0.10
No substrate	+0.22	+0.23	+0.44	+0.04	+0.66	+0.68	+0.10
	±0.10	±0.15	±0.14	±0.02	±0.29	±0.20	±0.03

glutamine synthetase according to Squires and Brosnan [16]. Labeled glutamine was purified by two passes over Dowex and purity of the products was confirmed by TLC, ion exchange chromatography and HPLC. Incorporation of label into the specific positions checked by hydrolysis with glutaminase followed by decarboxylation with glutamate decarboxylase (100% of the radioactivity recovered as CO_2 with 1-labeled substrate, 22% with U-labeled and $< 1\%$ with 5-labeled). 3-Mercaptopicolinate (3-MCPA), a specific inhibitor of phosphoenolpyruvate carboxykinase [3,13,14], was a gift from Dr. N.W. DiTullio, Smith, Kline and French, Philadelphia, PA, USA. Sodium acetoacetate was synthesized from acetyl acetoacetate according to the procedure of Krebs and Eggleston [17]. All other reagents were from standard sources as previously described [12–14].

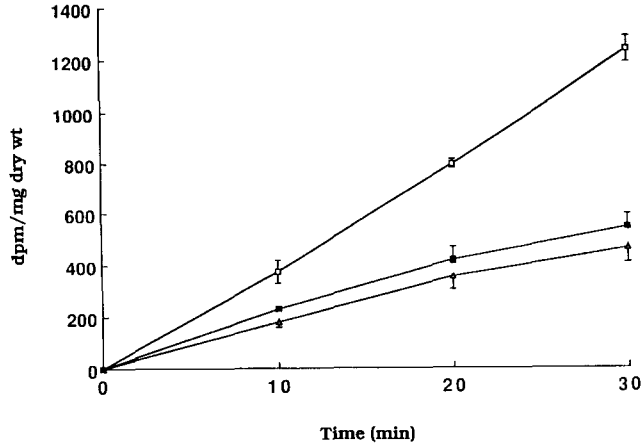

Fig. 1. Time-course of $^{14}CO_2$ production from labeled glutamine by rat enterocytes Enterocytes were incubated with L-glutamine (2 mM) labeled in the 1, 5 or U positions, as described in Section 2. Results are expressed as dpm in CO_2/mg dry wt cells, corrected for a specific activity of 25000 dpm/μmol glutamine and are means ± SEM for 4 enterocyte preparations. Substrate label; Δ, U; ■, 5; □, 1.

3. Results

The metabolism of glutamine by isolated rat enterocytes has been well documented and the results shown in Table 1 illustrate the magnitude of the changes in metabolites obtained in the present experiments. No changes in metabolite utilization or production from glutamine were seen when enterocytes were incubated with glutamine in the presence of dichloroacetate (1 mM) or sodium octanoate (1 mM) (not shown). Glutamine utilization was not affected by the presence of glucose, ketone bodies or 3-MCPA. The addition of glucose with glutamine increased the amount of alanine formed, with a slight decrease in glutamate accumulation and the addition of 3-MCPA resulted in a slight increase in aspartate accumulation. These findings are similar to those previously reported [12,13,18].

When differentially labeled glutamine was used the evolution of labeled CO_2 was linear with time up to 20 min. (Fig. 1). When the results are expressed relative to a common specific activity (dpm/μmol glutamine) it can be seen (Fig. 1, Table 2) that glutamine labeled in the 1 position gives rise to approx. 2.5-fold as much labeled CO_2 as that labeled uniformly, while the 5-labeled substrate results in approx. 1.2–1.4 times as much labeled CO_2 as the uniformly labeled substrate. The use of differentially labeled glutamine of similar specific activity allows a simple calculation of the extent of decarboxylation per glutamine molecule. For every five glutamine molecules the following labeled CO_2 yields are expected for two decarboxylations per molecule. From 1-labeled glutamine all five labeled carbons will be released in the initial decarboxylation to give 5-$^{14}CO_2$ but no labeled CO_2 will be produced at the second decarboxylation. With the 5-labeled substrate, the initial decarboxylation will not yield any labeled CO_2 but, due to the randomization of carbons 2 and 5 in the TCA cycle, half of the labeled carbon will be lost at the second decarboxylation yielding 2.5-$^{14}CO_2$. For the uniformly labeled substrate the specific activity of each carbon is one fifth that of the 1- and 5- labeled glutamine and two decarboxylations will yield 2-$^{14}CO_2$ from uniformly labeled glutamine. This will result in a $^{14}CO_2$ production ratio for 5:1:U labeled substrates of 2.5:1.25:1. This ratio was found experimentally (Table 2) and indicates that each glutamine molecule metabolized beyond glutamate undergoes two decarboxylations. The total amount of CO_2 produced and the ratio of label production (i.e., degree of decarboxylation) were not affected by the inclusion of glucose or 3-MCPA (Table 2).

To identify the three carbon end products of glutamine metabolism attempts were made to separate the products and determine the amount of incorporated glutamine carbon. The high amount of both

Table 2
Carbon dioxide production from labeled glutamine

	Label			
	[1-^{14}C]	[5-^{14}C]	[U-^{14}C]	1:5:U ratio
Additions				
None	826 ± 120	460 ± 22	326 ± 12	2.53:1.41:1
Glucose	842 ± 92	505 ± 75	367 ± 20	2.31:1.38:1
Ketone bodies	670 ± 50	368 ± 35	279 ± 11	2.40:1.32:1
3-MCPA	633 ± 88	414 ± 56	284 ± 28	2.23:1.46:1

Enterocytes were incubated for 20 min in the presence of 2 mM glutamine containing tracer amounts of glutamine labeled as shown. Glucose (10 mM), ketone bodies (3 mM β-hydroxybutyrate, 1 mM acetoacetate) or 3-MCPA (1 mM 3-mercaptopicolinate) were added as shown. Results are expressed as dpm in CO_2 per mg dry wt cells, corrected for a specific activity of 25000 dpm/μmol glutamine and are mean ± SEM for 4 enterocyte preparations. There was no significant difference in the values within any column.

Table 3
Fate of labeled glutamine carbon in isolated enterocytes.

Incubation	Metabolite (dpm)					
	Proline	Aspartate	Lactate	Pyruvate	Alanine	Total in C3 units
Glutamine	516	6629	1722 (27%)	65 (1%)	4710 (72%)	6497
Glutamine + Glucose (10 mM)	576	3863	4555 (72%)	546 (9%)	1188 (19%)	6289
Glutamine + 3-MCPA (1 mM)	543	7280	1184 (20%)	45 (1%)	4600 (79%)	5829

Enterocytes were incubated for 15 min as described in Section and 2 in the presence of 2 mM L-[U-^{14}C]glutamine (25000 dpm/μmol). Samples (100000 dpm) were separated by two-dimensional TLC and the results represent the radioactivity recovered in organic and amino acids. Numbers in parentheses represent the label recovered in that metabolite as a percentage of the total recovered in C3 units. A representative experiment (8.2 mg dry wt. enterocytes) is shown.

unlabeled and labeled glutamine and glutamate present in the samples caused difficulties with HPLC both in derivitization and detection and therefore TLC was used. However, the large amount of substrates present in the samples confounded the analysis. It was necessary to load considerable volumes of incubation medium to each plate in order to detect significant counts in the products and it was not possible to determine recoveries since ~ 90% of the label remained as glutamate and glutamine. A representative analysis is shown in Table 3 where the counts present in proline, aspartate, lactate, pyruvate and alanine were clearly separated on the plates. A small amount of glutamine carbon was always converted to proline and this was not altered by the presence of glucose or 3-MCPA. No detectable amounts of radioactivity were found in malate, citrate, fumarate, or α-ketoglutarate (not shown). Large amounts of radiolabeled glutamate and aspartate accumulated and the presence of glucose decreased the amount in aspartate but there was no consistent change noted in the presence of 3-MCPA. The total amount of radioactivity recovered in three carbon units (alanine, pyruvate and lactate) was not changed by the presence of glucose or 3-MCPA but the distribution of the label changed when glucose was present in the medium. In the presence of glutamine alone, with or without 3-MCPA, the distribution of label in lactate, pyruvate and alanine, was 27%, 1% and 72%, respectively, which correlates with the net production of three carbon compounds as 20% lactate, 7% pyruvate and 74% alanine (taken from Table 1). When glucose was present the distribution of the labeled products changed with the change in net production of three carbon compounds. The products and distribution of label were lactate (66%/72%), pyruvate (9%/9%) and alanine (26%/19%). These results mean that, given the equilibrium nature of both lactate dehydrogenase and alanine aminotransferase, any labeled pyruvate derived from glutamine is available for either lactate or alanine synthesis. When the magnitude of pyruvate to lactate flux is very high relative to that for alanine synthesis the majority of glutamine derived pyruvate is shunted into lactate and vice versa.

4. Discussion

Incubation of enterocytes with high concentrations of glutamine always results in the accumulation of large amounts of glutamate [14,18]. This is not seen in intestinal preparations in vivo where glutamine levels are much lower and is therefore an artifact of the in vitro experimental preparation [3]. This accumulation of glutamate is of no importance to the fate of glutamine carbon and this discussion will consider glutamine metabolism to mean that glutamine which is metabolized beyond glutamate. The results show that enterocytes are capable of two decarboxylations per glutamine molecule and that this is not changed by the addition of other substrates (glucose, fatty acids or ketone bodies) or by the use of inhibitors (3-MCPA) of phosphoenolpyruvate carboxykinase or activators (dichloroacetate) of pyruvate dehydrogenase. The first decarboxylation is presumably α-ketoglutarate dehydrogenase but the identity of the second step is not clear. The lack of effect with 3-MCPA would appear to rule out phosphoenolpyruvate carboxykinase/pyruvate kinase but there is always the possibility that the inhibitor was not able to inhibit the enzyme in these cells [3,12,13]. Other enzymes postulated to play a role are malic enzyme ($NADP^+$ requiring), a $NAD^+/NADP^+$-dependent malic enzyme and a direct oxaloacetate decarboxylase activity. These have been extensively discussed [3,5–8,12,13,19] and will not be considered further here.

The finding of incomplete glutamine oxidation is in agreement with previous work in enterocytes using both labeled and unlabeled substrates. Using tracer amounts of 2,3-^{14}C succinate in the presence of glutamine, Kelleher and colleagues [20] concluded that glutamine metabolism in enterocytes gave rise to three carbon products, lactate and alanine. These results were questioned since many cells show restricted permeability to succinate and the use of long (60 min. incubation periods decreases the viability of isolated enterocytes [12]. However, Kelleher and coworkers confirmed that succinate was taken up by enterocytes and can therefore be used to model glutamine metabolism [21]. Windmueller and Spaeth [5–8] found 55–65% of the glutamine carbon metabolized by the intact small intestine as carbon dioxide, a result indicative of three decarboxylations and clearly contrary to

the results found in isolated enterocytes. Two possible explanations for the results of Windmueller and Spaeth are that further metabolism of the three carbon end product of enterocyte glutamine metabolism occurs in other cells of the intestine, or that the experiments overestimate the amount of glutamine going to CO_2. The possibility of further metabolism by other cells is highly unlikely since there is no evidence of a two-carbon compound (or one which is synthesized from two carbons, such as lipid) as a major end product of intestinal glutamine metabolism. The possibility of overestimation of CO_2 production by Windmueller and Spaeth has been discussed by Hartman and Plauth [11] who proposed that some CO_2 release could occur due to exchange reactions due to flux through pyruvate carboxylase (present in enterocytes) and the TCA cycle. The most likely explanation is a combination of a low rate of complete oxidation of pyruvate derived from glutamine and considerable experimental error in results based on the use of solely U-labeled substrate. The use of differentially labeled substrates in the current study allows the unequivocal conclusion that enterocytes oxidize glutamine through two decarboxylations.

The results are also in agreement with many other glutamine utilizing cells such as immunocytes and cells in tissue culture which show a truncated TCA cycle with regard to glutamine metabolism [1–3]. The end products are either lactate or aspartate, with alanine being rarely found since alanine aminotransferase activity is invariably very low in such cells. Whether or not a four (aspartate) or three carbon (lactate) end product is produced probably also reflects the lack of enzymatic mechanisms for the production of pyruvate from four carbon substrates. However, there may also be selective use of endogenous substrates since, in tumor mitochondria, malate derived from glutamine is preferentially converted to oxaloacetate while exogenous malate undergoes decarboxylation via a $NAD^+/NADP^+$-linked malic enzyme [22]. The fate of glutamine derived carbon is therefore not only cell-specific but also depends on the presence or absence of other substrates [23]. In isolated enterocytes the results presented in Table 3 show that the distribution of glutamine carbon varies with the relative rate of flux through the pyruvate pool. When glucose is absent and glycolytic flux is low most the glutamine carbon metabolized to pyruvate will be found in alanine, however when glycolytic flux is increased more than 10-fold (by the addition of glucose) then the amount of glutamine carbon passing through pyruvate is unchanged but it is now found predominantly as lactate. Thus the results indicate that pyruvate derived from glutamine and glucose are present as one pool in these cells and the fate of this pyruvate depends on the relative rates of lactate and alanine formation.

The results are of considerable significance to the provision of gluconeogenic precursors in vivo. At times of high gluconeogenesis to maintain blood glucose homeostasis there is a need for the body to conserve gluconeogenic potential (three carbon compounds). Since enterocytes utilize ketone bodies and spare glutamine metabolism in both starvation and uncontrolled streptozotocin diabetes it was proposed [12] that this was a mechanism, similar to the glucose sparing effects of fat derived fuels, to spare glutamine for hepatic gluconeogenesis. However, the results presented in this paper show that enterocytes always conserve the gluconeogenic potential of glucose by restricting the extent of decarboxylation. Therefore the function of sparing of glutamine metabolism in the intestine is not obvious. It may allow more glutamine for renal gluconeogenesis (and ammonia formation important in acid-base balance) or it maybe related to the need to reduce the metabolic demands on the liver. As Halperin and colleagues [24,25] have established, hepatic amino acid catabolism is an ATP generating process, and to balance this gluconeogenesis (and urea synthesis) must occur. Thus under some conditions, gluconeogenesis can be viewed as a system allow the catabolism of excess amino acids by regeneration of ADP, rather than as a mechanism for the maintenance of blood glucose levels. The prior metabolism of glutamine from five carbons to three carbons by the intestine not only provides considerable energy for the intestine but also reduces the ATP generating potential arriving at the liver. This maybe important in the fed state, when the liver receives large amounts of amino acids but maynot be so important in starvation, diabetes, and other conditions where gluconeogenesis operates to maintain blood glucose homeostasis.

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