

Glutamine and glutamate—their central role in cell metabolism and function

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Glucose is widely accepted as the primary nutrient for maintenance and promotion of cell function. However, we propose that the 5-carbon amino acids, glutamine and glutamate, should be considered to be equally important for maintenance and promotion of cell function. The functions of glutamine are many and include: substrate for protein synthesis, anabolic precursor for muscle growth, acid–base balance in the kidney, substrate for ureogenesis in the liver, substrate for hepatic and renal gluconeogenesis, an oxidative fuel for intestine and cells of the immune system, inter-organ nitrogen transport, precursor for neurotransmitter synthesis, precursor for nucleotide and nucleic acid synthesis and precursor for glutathione production. Many of these functions are connected to the formation of glutamate from glutamine. We propose that the unique properties regarding concentration and routes of metabolism of these amino acids allow them to be used for a diverse array of processes related to the specialized function of each of the glutamine utilizing cells. In this review we highlight the specialized aspects of glutamine/glutamate metabolism of different glutamine-utilizing cells and in each case relate key aspects of metabolism to cell function. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS— glutamine; glutamate; metabolism; cell function

INTRODUCTION

The physiological importance of the amino acid L-glutamine for promoting and maintaining cell function is now widely accepted. The importance of glutamine to cell survival and proliferation *in vitro* was first reported by Ehrensvar *et al.* in 1949¹ but was more fully described by Eagle *et al.* in 1956.² Glutamine had to be present at 10- to 100-fold in excess of other amino acids in culture and could not be replaced by glutamic acid or glucose. This work led to the development of the first tissue culture medium that contained essential growth factors, glucose, a mixture of 19 essential and non-essential amino acids at approxi-

mately physiological concentrations and a high concentration of glutamine (2 mmol l⁻¹).

It is now known that a large number of tissues and cells of the body utilize glutamine at high rates and that glutamine utilization is essential for their function. These tissues and cells include kidney, intestine, liver, specific neurons in the CNS, cells of the immune system and pancreatic β -cells (see references 3 and 4 for further details).

L-Glutamine is important as a precursor for peptide and protein synthesis, amino sugar synthesis, purine and pyrimidine and thus nucleic acid and nucleotide synthesis, as well as providing a source of carbons for oxidation in some cells. However, the immediate product of glutamine metabolism in most cells is L-glutamate which is produced by the action of glutaminase, an enzyme found at high concentration and associated with the mitochondria in cells which readily utilize glutamine. L-Glutamate is the most abundant

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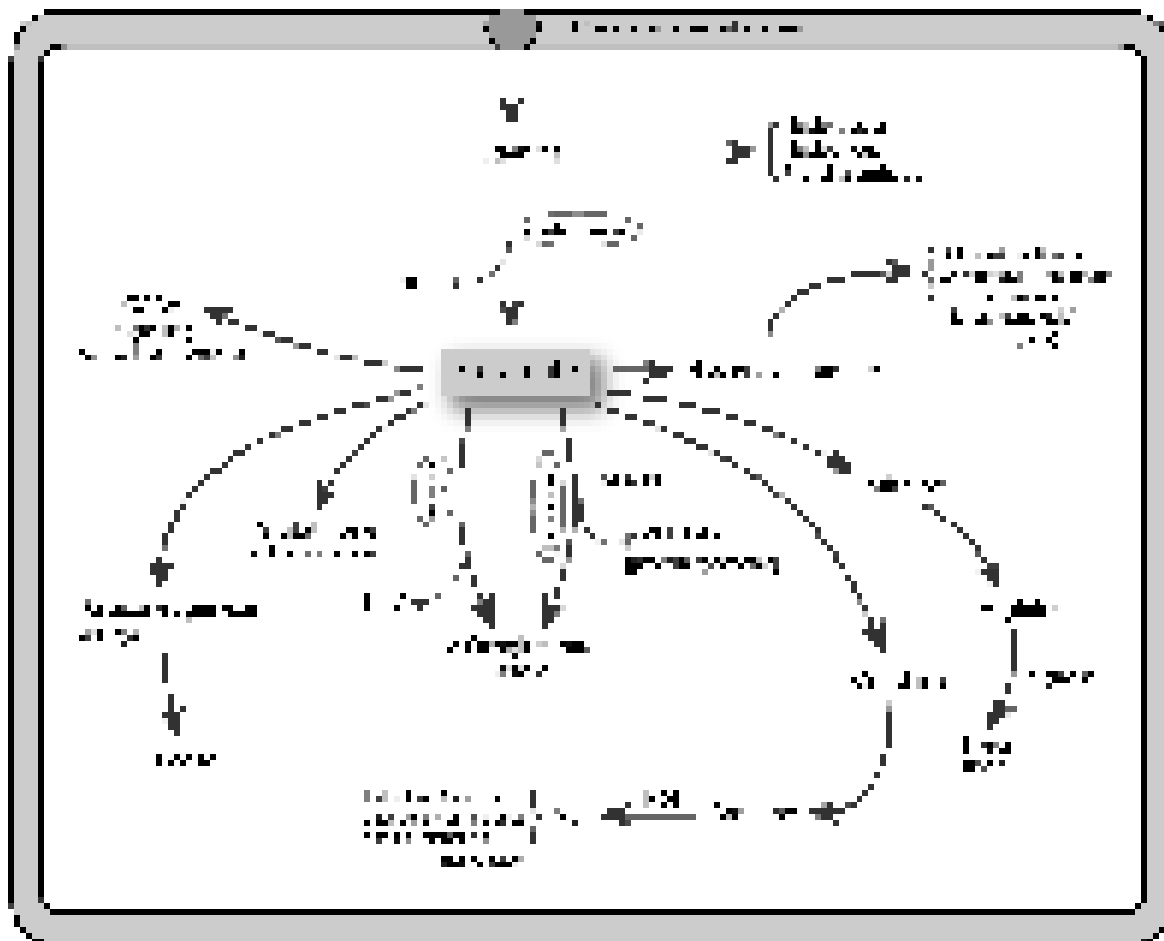


Figure 1. Overview of glutamine and glutamate metabolism in mammalian cells. Glutamate is produced from glutamine through glutaminase activity. Glutamate can then be converted into γ -amino butyric acid (GABA), ornithine, 2-oxoglutarate, glucose or glutathione. The probable functions of the glutamate products are indicated as well as the cells or organs where the metabolic pathway preferentially occurs

intracellular amino acid (reported concentrations vary between 2 and 20 mM) whereas L-glutamine is the most abundant extracellular amino acid *in vivo* (0.7 mM compared to an approximate L-glutamate concentration of 0.02 mM). L-Glutamate cannot readily traverse cell membranes as it has an overall charge -1 at pH 7.4 and amino acid transporters capable of transporting glutamate into the cell are found at low density in the plasma membrane with the exception of specialized glutamate-metabolizing cells located in the CNS,⁵ liver,^{6,7} intestine^{8,9} and kidney.¹⁰ L-Glutamate appears to be at the crossroads of amino acid metabolism, where it can donate its amino group for new amino acid synthesis (transamination) or can

lose the amino group, as NH_4^+ , via deamination to 2-oxoglutarate (see Figure 1). In some tissues and cells such as liver, skeletal muscle or astrocytes, glutamate and NH_3 may be combined by the action of glutamine synthetase, to produce glutamine. This glutamine is then exported from the cell.

L-Glutamine is required for a number of specific biochemical reactions, outlined above. However, of greater physiological importance to many cells, is the fact that L-glutamine is a precursor of L-glutamate. This review will highlight the critical role of L-glutamine and L-glutamate metabolism for maintenance and promotion of cell function in a diverse selection of cell types.

GLUTAMINE/GLUTAMATE IN THE KIDNEY

Glutamine is quantitatively the most important donor of NH_3 in the kidney. The NH_3 is cleaved from glutamine by the action of phosphate-dependent glutaminase, the expression of which is subject to pH regulation.¹¹ NH_3 is exported to the lumen of the collecting tubule where it combines with exported H^+ to form NH_4^+ which is lost to the urine. H^+ is created from carbonic acid which dissociates to form HCO_3^- and H^+ . HCO_3^- subsequently enters the circulation where it is important for maintenance of blood pH. Therefore glutamine metabolism in the kidney is essential for acid-base buffering in the plasma.^{11,12} The carbon skeleton of glutamate in the kidney, created by the action of glutaminase, is converted via formation of 2-oxoglutarate, succinate, fumarate, malate and oxaloacetate to phosphoenolpyruvate (or malate to pyruvate directly) and then into the pathway of gluconeogenesis (Figure 2). Glucose produced by this pathway provides up to 25% of circulating plasma glucose *in vivo*.¹³

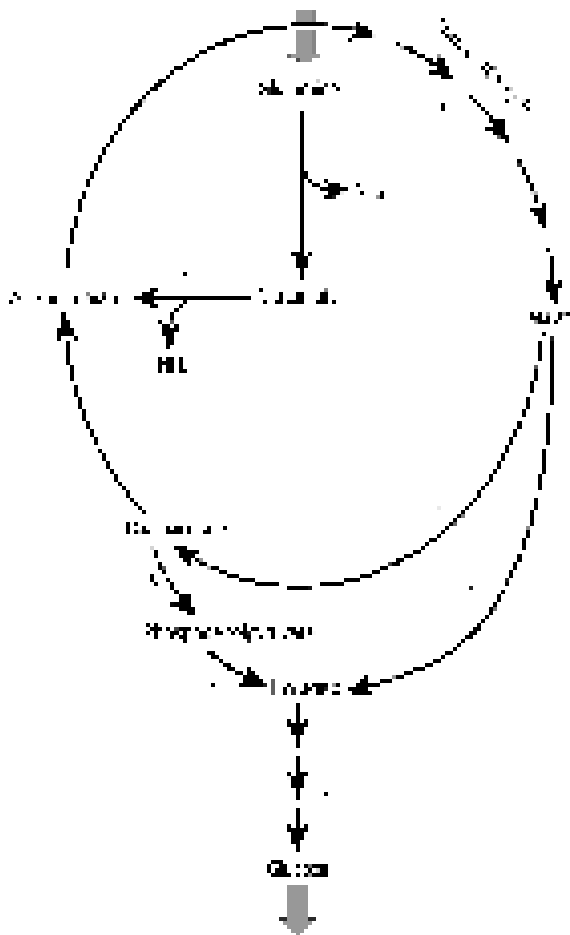


Figure 2. Pathway of glutamine metabolism in the kidney. 1, Phosphate-dependent glutaminase; 2, glutamate dehydrogenase; 3, reactions of TCA cycle; 4, NADH-malate dehydrogenase; 5, NADP^+ -dependent malic enzyme; 6, phosphoenolpyruvate carboxykinase; 7, pyruvate kinase; 8, pathway of gluconeogenesis (cytosol)

GLUTAMINE/GLUTAMATE IN THE INTESTINE

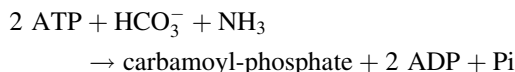
Glutamine is quantitatively the most important fuel for intestinal tissue. It is metabolized to L-alanine by a route involving conversion to glutamate, then 2-oxoglutarate via glutaminase and glutamate dehydrogenase respectively, then TCA cycle conversion to malate (2-oxoglutarate, succinate, fumarate and finally malate) followed by the action of NADP^+ -dependent malic enzyme to create pyruvate which undergoes amination to produce L-alanine via the action of alanine transaminase (Figure 3).

The NADH and FADH_2 generated via this pathway are used for electron donation to the electron transport chain in the mitochondria and thus they promote ATP synthesis. The L-alanine produced in this pathway is exported to the hepatic portal vein for transport to the liver.¹⁴

GLUTAMINE/GLUTAMATE IN THE LIVER

The liver is the central site for nitrogen metabolism in the body.¹⁵ Nitrogen is transported from peripheral tissues (principally from muscle and lung) to the central organs as glutamine and alanine (if the glutamine is taken up and metabolized by the intestine).³ Glutamine can be cleaved by glutaminase to yield glutamate and NH_3 . The mitochondrial carbamoyl

phosphate synthetase (CPS I) then can catalyse the following reaction:



The enzyme is allosterically activated by N-acetylglutamate and may thus be indirectly regulated by glutamate concentration. Carbamoyl phosphate may combine with ornithine in the urea cycle to produce citrulline, which is subsequently converted to arginosuccinate and then arginine (Figure 4). Arginine is subsequently cleaved by arginase to produce urea and ornithine. In mammalian tissues another isoform

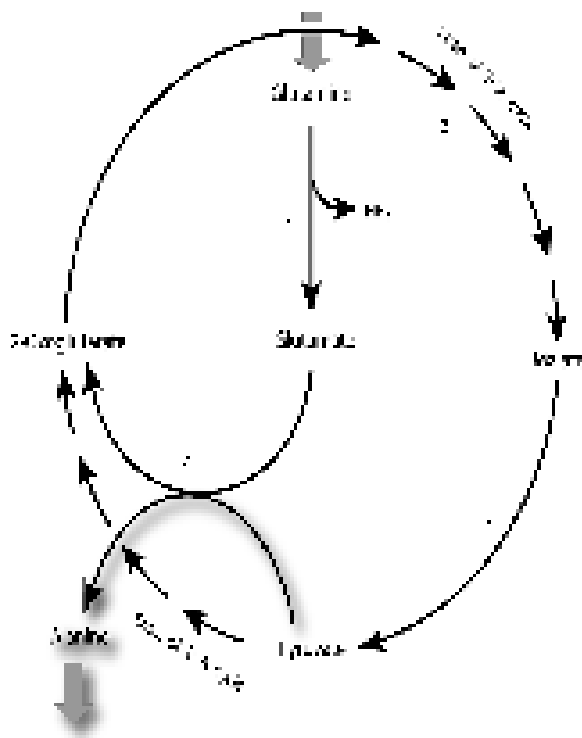
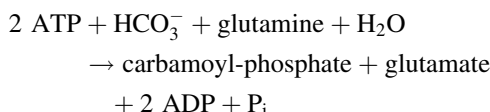


Figure 3. Pathway of glutamine metabolism in the intestine. 1, Phosphate-dependent glutaminase; 2, alanine aminotransferase; 3, reactions of the TCA cycle; 4, NADP⁺-dependent malic enzyme

of CPS exists, termed CPS II. This is a large multifunctional cytosolic protein¹⁶ that catalyses formation of carbamoyl phosphate:



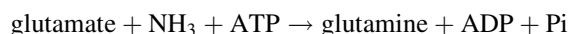
The reaction also provides the source of the N3 atom of pyrimidine nucleotides whereas the amide of glutamine is used directly for the source of the N3 and N9 atoms of purines.

Glutamine metabolism is zoned in the liver so that glutamine is taken up by the periportal cells of the liver in which there is a relatively high glutaminase activity and the ammonia produced directed towards carbamoyl phosphate synthesis.^{17,18}

Glutamate that has been produced in the periportal cells may be further metabolized to produce other amino acids by transamination or may enter the TCA cycle as an anaplerotic substrate or may enter the pathway of gluconeogenesis via formation of phosphoenolpyruvate from oxaloacetate (Figure 4).

Thus gluconeogenesis from glutamine may be a major consumer of glutamate-derived carbon in the liver, resulting in the formation and export of glucose.¹⁹

Glutamine formation and release from the liver, on the other hand, occurs principally in the perivenous region (Figure 4). The hepatocytes in this area are rich in glutamine synthetase.¹⁹ The substrate(s) for glutamine synthesis are of course glutamate and NH₃. Glutamate may be produced via glucose conversion to 2-oxoglutarate and subsequent conversion to glutamate via glutamate dehydrogenase. However, recent data have suggested that arginine catabolism may provide glutamate for the glutamine synthetase reaction.²⁰ The glutamine synthetase reaction is energy requiring and is described below:



GLUTAMINE/GLUTAMATE IN THE CNS

The major transmitters at excitatory synapses in the central nervous system are glutamate and acetylcholine whereas inhibitory signals are carried by glycine and γ -amino butyric acid.^{21,22} The existence of a glutamine–glutamate cycle in the CNS has recently been confirmed.²³ Glutamine is synthesized from glutamate in the astrocytes so as to return the glutamate that is removed from the synaptic cleft after release from the presynaptic neuron. The neuron will readily convert the astrocyte-derived glutamine to glutamate via glutaminase, to complete the cycle. The cycle is energy dependent as ATP is consumed in the synthesis of glutamine from glutamate. In the human cortex the cycle appears to account for 80% of the energy derived from glucose oxidation.^{24,25}

GLUTAMINE/GLUTAMATE IN CELLS OF THE IMMUNE SYSTEM

It is now widely accepted that glutamine is utilized at high rates by isolated cells of the immune system such as lymphocytes, macrophages and neutrophils^{26–28} (Table 1). Although the activity of the first enzyme responsible for the metabolism of glutamine, phosphate-dependent glutaminase, is high in these cells, the rate of oxidation is low. Much of the glutamine is converted to glutamate, aspartate (via TCA cycle activity), lactate and under appropriate conditions, CO₂ (Table 1).

Glutamine has been reported to enhance many functional parameters of immune cells such as T-cell proliferation, B-lymphocyte differentiation, macrophage phagocytosis, antigen presentation and

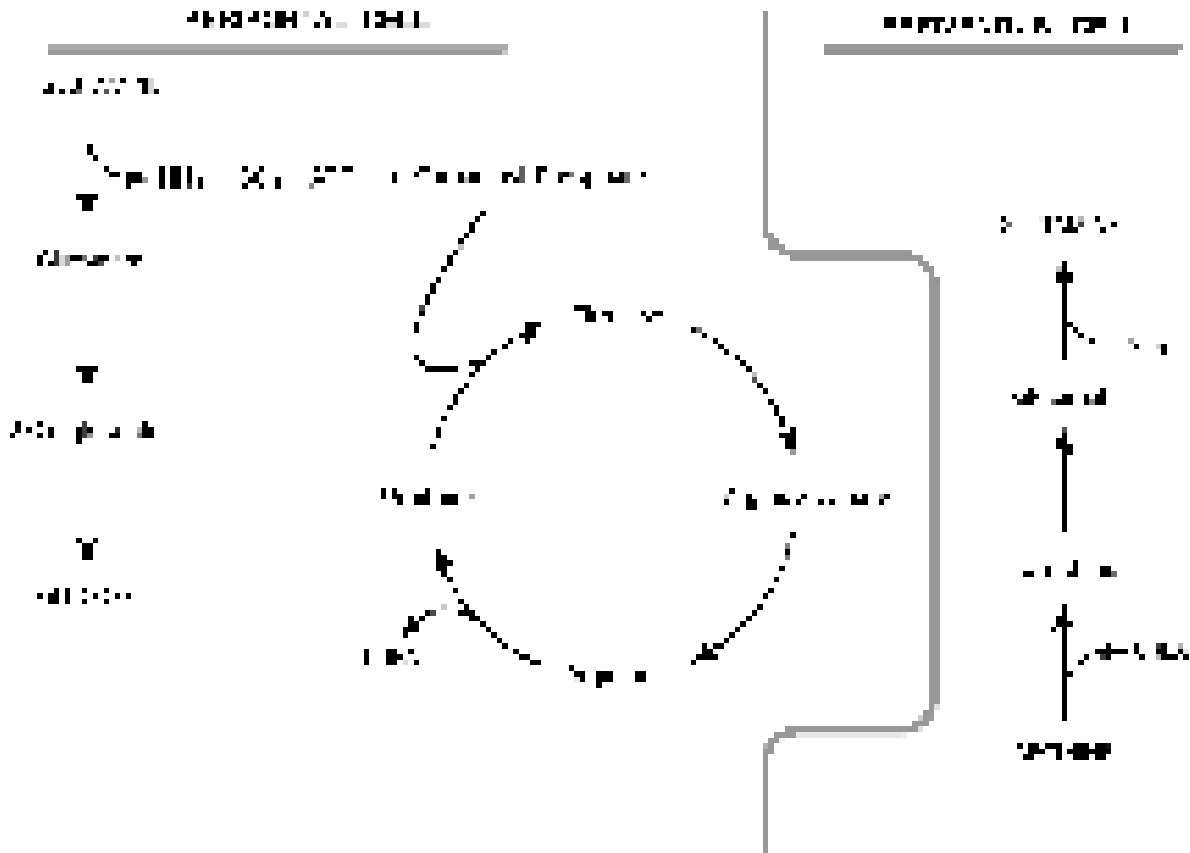


Figure 4. Pathway of glutamine metabolism in the periportal and perivenous cells of the liver. Glutamine nitrogen is directed to urea synthesis while carbon is directed to glucose synthesis in the periportal cells. In conditions in which arginine availability is not limiting, glutamine is synthesized in the perivenous cells

cytokine production^{29–33} plus neutrophil superoxide production and apoptosis (T.C. Pithon-Curi *et al.*, unpublished data).³⁴

Although glutamine may be required by these cells as a precursor for nucleic acid and nucleotide synthesis, the provision of glutamate may be equally important in cells of the immune system (note the high glutamate/glutamine ratio after 1 h incubation in the

presence of glutamine, Table 1). Glutamate is involved in a number of key functions, in addition to amino acid transamination, in lymphocytes, macrophages and neutrophils. Provision of NADPH, via action of NADP⁺-dependent malic enzyme, which catalyses the conversion of malate (which is derived from glutamate via formation of 2-oxoglutarate, succinate, and fumarate) to pyruvate, may be one of its

Table 1. Rates of glutamine utilization and of lactate, glutamate and ¹⁴CO₂ production by isolated incubated mouse macrophages, rat lymphocytes or rat neutrophils

Addition to incubation medium	Glutamine utilization	Lactate production	Glutamate production	¹⁴ CO ₂ production	Glutamate/glutamine
Mouse macrophages	-186	33	137	9	0.74
Rat lymphocytes	-223	9	132	6.1	0.59
Rat neutrophils	-770	320	250	6.5	0.31

Data are taken from Ardawi and Newsholme,²⁶ Newsholme *et al.*²⁷ and Pithon-Curi *et al.*²⁸ Units: nmol h⁻¹ mg⁻¹ protein. The minus sign (-) indicates utilization. Glutamate/glutamine refers to the ratio of glutamate production to glutamine utilization.

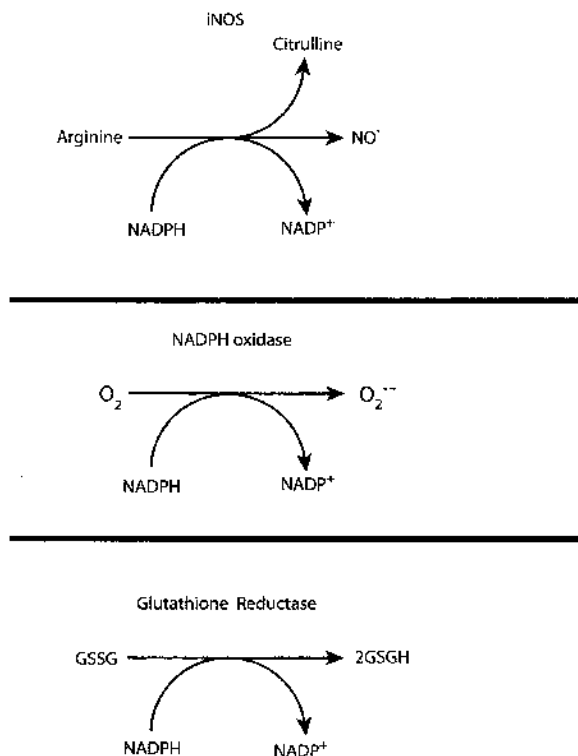


Figure 5. Common intracellular reactions utilizing NADPH. Top panel, inducible nitric oxide synthase (iNOS); middle panel, NADPH oxidase; bottom panel, glutathione reductase

functions.³⁵ NADPH is required for biosynthetic reactions such as fatty acid synthesis or for production of free radicals such as O₂^{•-} or NO by the NADPH oxidase and iNOS respectively (reference 29, and Figure 5). NADPH is also required for glutathione reductase activity and as such plays an important role in increasing reduced glutathione concentration and hence antioxidant defenses and delay in apoptosis via stabilization of neutrophil mitochondria (reference 36 and Figure 5). Indeed the greater proportion of glutamine metabolized to lactate in the neutrophil compared to the macrophage or lymphocyte may be due to significantly higher demands for NADPH in the neutrophil. The quantitative importance of the key NADPH-generating enzyme, NADPH-dependent malic enzyme, which would form part of the glutamine → lactate pathway has been demonstrated previously.³⁷ It was estimated that the rates of generation of NADPH from malic enzyme (55 nmol h⁻¹ 10⁻⁶ cells) and the pentose phosphate pathway (59 nmol h⁻¹ 10⁻⁶ cells) were approximately equal when phagocytes were incubated in appropriate conditions.³⁷

Glutamate is also required as a precursor for ornithine synthesis in macrophages and monocytes. This pathway connects with the urea cycle via synthesis of citrulline catalysed by ornithine carbamoyl transferase (macrophages and monocytes also express significant carbamyl phosphate synthase activity³⁸) and ultimately results in formation of arginine and thus substrate for iNOS.³⁸ Extracellular arginine is depleted by active secretion of the enzyme arginase by macrophages and monocytes, and these cells subsequently become dependent on intracellularly derived arginine for NO synthesis.³⁸ Glutamate may also serve as a precursor for glutathione synthesis and as such may play a direct role in antioxidant defenses³⁹ in these cells (Figure 1). Indeed γ -glutamylcysteine synthetase, the rate-determining enzyme in GSH synthesis, utilizes cellular glutamate as a substrate and thus will respond to an elevation of cellular glutamate concentration by increased activity.

GLUTAMINE/GLUTAMATE IN THE PANCREATIC β -CELL

Insulin secretion from the cell type responsible for its production and release, the pancreatic β -cell, is tightly regulated due to the potent effect of insulin on promotion of anabolic carbohydrate, lipid and protein metabolism in liver, adipose and muscle as well as many other target tissues. Glucose is the primary insulin secretagogue in the pancreatic β -cell (located in the endocrine islets of Langerhans). However, glutamine has been reported to weakly enhance glucose-stimulated insulin secretion from pancreatic β -cells but does not promote insulin secretion by itself due to tight regulation of glutamate dehydrogenase activity.^{40,41} Glutamine may act as an anaplerotic substrate in the β -cell, via formation of glutamate and 2-oxoglutarate, subsequently stimulating a catalytic enhancement of glucose oxidation.⁴² Nutrient metabolism is intimately connected with the process of insulin secretion from the β -cell. Nutrient metabolism results in an increase in the ATP/ADP ratio, a closure of K_{ATP}⁺ channels, membrane depolarization, opening of voltage-dependent Ca²⁺ channels (VDCC), an increase in cytosolic Ca²⁺ concentration and promotion of insulin exocytosis.⁴³ The mitochondria play a critical role, via oxidative phosphorylation, in increasing the ATP/ADP ratio. However, the mitochondria are also important for generation of metabolic coupling factors that act to further enhance insulin secretion in a K_{ATP}⁺ channel-independent manner.^{44,45} One of these metabolic coupling factors has been identified as glutamate.^{46,47} However, the mechanism by

which glutamate stimulates insulin secretion has yet to be elucidated.

Glutamate is additionally important in the β -cell as a substrate for the enzyme glutamic acid decarboxylase, which produces the signalling molecule γ -amino butyric acid (GABA⁴⁸). GABA production and secretion may be important for regulation of insulin secretion in the intact islet of Langerhans.⁴⁹

Recent reports have highlighted the important regulatory role of glutamate dehydrogenase in the β -cell. Mutations in the GTP allosteric site within the enzyme, which result in a lower affinity for the allosteric inhibitor GTP, have been shown to result in elevated insulin secretion and associated hyperinsulinaemia (thus leading to hypoglycaemia) in affected individuals.^{50–52} A recent paper by Brennan *et al.*, 2002⁵³ has provided a mechanistic explanation for the synergistic effect of L-alanine on glucose stimulated insulin secretion. L-Alanine oxidation was absolutely required for the synergistic effect on glucose-stimulated insulin secretion and was additionally accompanied by an elevation in intracellular glutamate concentration. Thus the metabolic importance of glutamate concentration and glutamate dehydrogenase activity with respect to insulin secretion in the β -cell is now firmly established. However, the metabolic interplay between glucose, ATP, ADP, glutamate dehydrogenase activity, glutamine, glutamate plus additional metabolites (such as malonyl-CoA) and the implication for regulation of β -cell insulin secretion has yet to be fully determined.⁵⁴

CONCLUDING REMARKS

Glutamine is the most abundant amino acid found in blood plasma.⁵⁵ It is a major transporter of nitrogen from sites of glutamine synthesis (skeletal muscle, liver, lung) to sites of utilization, including kidney, intestine, neurons, cells of the immune system and, under appropriate conditions of acid–base balance, liver.⁵⁶

Given the importance of plasma glutamine to cell function, it is not surprising that dietary supplementation or parenteral nutrition can improve the outcome for critically ill patients, post-surgical patients or those recovering from injury.^{29,57} The rationale for glutamine supplementation is based on evidence that muscle glutamine content falls due to enhanced muscle glutamine release but plasma glutamine is decreased due to greatly elevated glutamine demand in such patients.⁵⁸

Glutamine itself may act as a key precursor for nucleic acids and nucleotides in glutamine-consuming

cells, but in many physiological circumstances acts to provide glutamate, which appears to promote a wider array of metabolic functions compared to glutamine (Figure 1).

Ultimately glutamine and glutamate metabolism are exquisitely related to the function of the glutamine-requiring cell, for example provision of NH₃ for acid buffering and carbon for glucose production in the kidney, partial oxidation and alanine production in the intestine, provision of NH₃ for urea synthesis and carbon for glucose production in the liver, neurotransmitter synthesis in the brain, NADPH and free radical production plus antioxidant defenses, as well as DNA and protein synthesis in cells of the immune system, and metabolic coupling factors that synergistically promote insulin secretion from the pancreatic β -cell. The pathways of glutamine and glutamate metabolism have adapted to cater for the unique function of the glutamine-utilizing cell (Figures 2–4) and thus could not be replaced by other metabolic inputs if they fail. In this respect we should consider glutamine and glutamate metabolism to be as important as glucose metabolism in the cell, due to the wide variety of metabolic roles undertaken that are critical to cell function.

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