2 Aminotransferases

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2.1 Abstract

The aminotransferases are PLP-dependent proteins which catalyse the transfer of an amino group from the donor amino acid to α-ketoglutarate, forming glutamate and the respective keto acids. Several key aminotransferase proteins have been identified as playing central roles in whole-body nitrogen metabolism, where they share common functions as nitrogen donors. These pathways play integrated roles within cells and between tissues, shuttling metabolites alluding to distinct pockets of compartmented metabolic activity. These anaplerotic shuttles interface with key metabolic pathways, for example the glutamate/glutamine cycle and TCA cycle, facilitating the regeneration of key metabolites such as the primary neurotransmitter glutamate. Contributions to glutamate levels in the brain from these anaplerotic pathways exceed 30%, illustrating their importance in maintaining the neurotransmitter pool of glutamate in neuronal cells. Knowledge of these pathways is not only important to our understanding of normal physiological mechanisms, but even more to the ways in which they alter and contribute to the pathogenesis of disease. The pathological implications of impaired aminotransferase metabolism is discussed, in particular their potential role in glutamate toxicity, which has been implicated in the pathogenesis of neurodegenerative disease. Finally, because of their tissue distribution these proteins have additional roles as biomarkers of disease, and can be used in the differential diagnosis of acute and chronic hepatic injury.

2.2 Introduction

The compartmentation of metabolic substrates and the subcellular localization of enzymes contribute to a fascinating interplay of highly regulated pathways. Several metabolic pathways, which play central roles in protein metabolism, are governed by the aminotransferases. Although numerous aminotransferase proteins exist, this chapter details the whole-body distribution of several specific aminotransferases, with particular focus on their role in anaplerotic pathways to generate and maintain the pool of brain glutamate through their involvement in metabolic shuttles. Furthermore, their role in disease is discussed with particular reference to the clinical application of serum aminotransferases.

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Transamination reactions are facilitated by pyridoxal phosphate-dependent (PLP) transaminase enzymes (Christen and Metzler, 1985). The key aminotransferase proteins discussed here include the branched chain aminotransferases (BCAT) [E.C. 2.6.1.42], the alanine aminotransferases (ALT) [glutamate pyruvate transaminase or alanine 2-oxo-glutarate E.C. 2.6.1.2], and the aspartate aminotransferase proteins (AST) [glutamic oxaloacetic transaminase or L-aspartate:2-oxoglutarate aminotransferase, E.C. 2.6.1.1]. These proteins have a mitochondrial and cytosolic isoform with tissue-specific locations. The crystal structures of BCAT and AST have been elucidated, where the catalytically competent structure of these aminotransferases is a homodimer (Jansonius *et al.*, 1984a,b; Yennawar *et al.*, 2002, 2006; Goto *et al.*, 2005). These enzymes belong to a large family of homologous proteins which operate by the same basic mechanism (Jansonius, 1998). PLP-dependent enzymes have been classified into four families with different fold types based on their three-dimensional structures (Jansonius, 1998; Mehta and Christen, 2000; Salzmann *et al.*, 2000; Schneider *et al.*, 2000). With the exception of the BCAT proteins which fall into the fold-type IV class of proteins, most of the PLP aminotransferases have been placed in the fold type I or L-aspartate aminotransferase family. A unique feature of the fold type IV family is that the proton is abstracted from the C4′ atom of the coenzyme-imine or external aldimine on the re face instead of the si face of the PLP cofactor (Yoshimura *et al.*, 1996).

2.2.1 Transamination

The mechanism of transamination consists of the coupled half-reaction in which the PLP cofactor transfers between its PLP and pyridoxamine (PMP) form (Fig. 2.1) (Karpeisky and Ivanov, 1966; Ivanov and Karpeisky, 1969; Kirsch *et al.*, 1984). The substrates of the forward reaction are dictated by the specificity of the particular transaminase, whereas L-glutamate features in the reverse reaction for most transaminases. The cofactor PLP is covalently attached to the enzyme via a Schiff base linkage, as a result of the condensation of its aldehyde group with the ε-amino group of a lysine residue. Transamination occurs via a Ping-Pong Bi-Bi mechanism, where each half reaction is divided into three stages. Interaction of the transaminases with their respective substrates involves the nucleophilic attack of the α-amino group of amino acid 1 (e.g. isoleucine) with the enzyme-PLP Schiff base carbon atom to form an amino acid-PLP Schiff base (external aldimine) with release of the enzyme (e.g. BCAT) (Fig. 2.1a). After transamination the second step is keto-enol tautomerism which involves the interconversion between the keto-enol form resulting in the formation of an α-keto acid-PMP Schiff base. This is subsequently hydrolysed to PMP and an α-keto acid, the final step in the first half-reaction (Fig. 2.1b). The second half reaction involves the same three steps but in reverse. In this stage the substrate is a second α-keto acid which reacts with PMP forming a Schiff base. Tautomerization facilitates the conversion from α-keto acid PMP to amino acid 2-PLP Schiff base. Subsequently, an internal aldimine is formed through attack from the ε-amino group of the active site lysine, leading to the release of a new amino acid and the regeneration of the aminotransferase.

The BCAT enzymes catalyse the transfer of the α-amino group from the hydrophobic branched chain amino acids (BCAA) leucine, isoleucine, and valine to α-ketoglutarate, releasing their respective keto acids: ketoisocaproate, keto methyl valerate, and ketoisovaline, and glutamate, regenerating the enzyme (Ichihara and Koyama, 1966; Taylor and Jenkins, 1966a,b,c). Both ALT and AST operate by the same basic mechanism generating pyruvate and glutamate, and oxaloacetate and glutamate, respectively (Glinghammar *et al.*, 2009). The transamination reaction of each aminotransferase is summarized in Box 2.1.

2.2.2 Cellular distribution of aminotransferases

Although aminotransferase activity is found in most tissues, each isoform shows tissue specificity with distinct subcellular location, which points to distinct functional roles

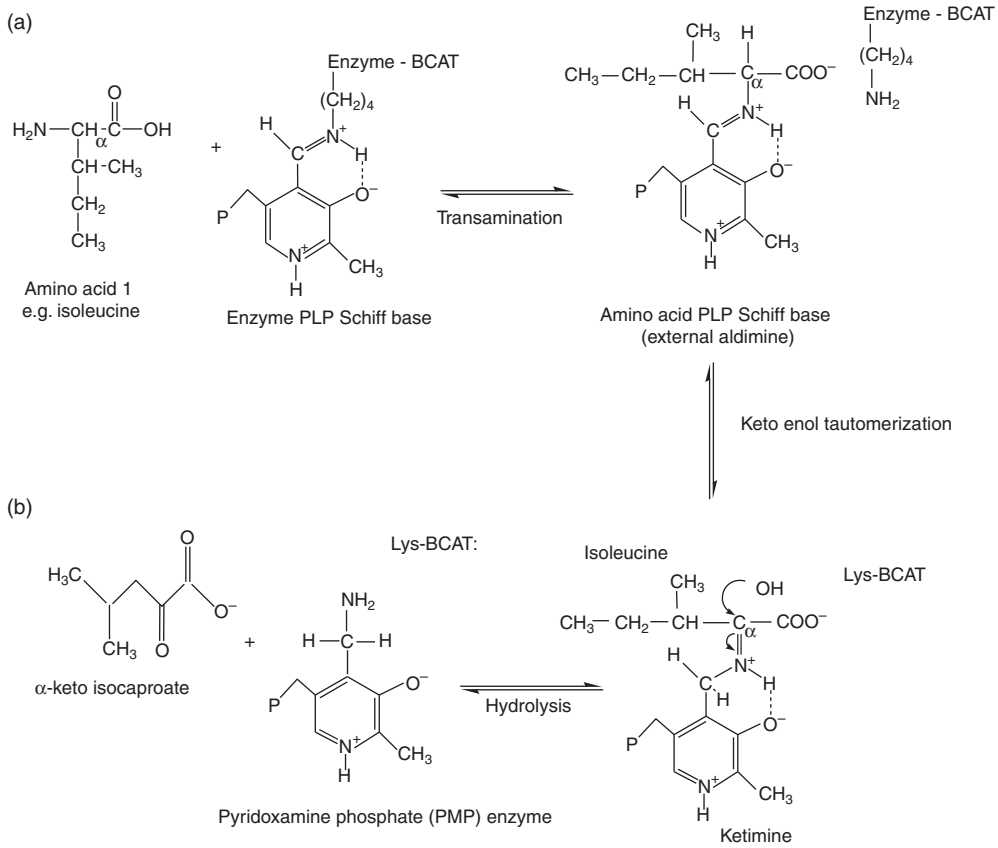

Fig. 2.1. Transamination of the branched chain amino acid isoleucine (first half reaction). (a) The enzyme–PLP Schiff base carbon undergoes a nucleophilic attack by the α-amino group of isoleucine resulting in the formation of an amino acid–PLP Schiff base and the release of BCAT. (b) Following keto enol tautomerization the resulting ketamine undergoes hydrolysis generating the α-keto acid, α-keto isocaproate, and the PMP form of BCAT.

Box 2.1. Transamination reactions of the aminotransferase proteins.

L-Leucine + α-ketoglutarate	BCAT	α-Ketoisocaproate + L-glutamate
L-Valine + α-ketoglutarate	BCAT	α-Ketoisovalerate + L-glutamate
L-Isoleucine + α-ketoglutarate	BCAT	α-Keto-β-methylvalerate + L-glutamate
L-Aspartate + α-ketoglutarate	AST	oxaloacetate + L-glutamate
L-Alanine + α-ketoglutarate	ALT	pyruvate + L-glutamate

of these proteins in tissues. The cellular distribution of these enzymes was first characterized in murine and rat models and has since progressed to human and cell systems. These models have offered enumerable insights into the compartmentation of many metabolic pathways, and indeed the flow of metabolites between these compartments. Understanding the differential distribution of these proteins and the factors which contribute to their regulation is pivotal to our understanding of the pathogenesis of disease, as many specific enzymes or pathways of metabolism are altered in adverse conditions.

2.2.2.1 Cellular distribution of the BCAT proteins

In mammals there are predominantly two BCAT isoforms, encoded by two different genes, which show both tissue and cellular-specific locations. Although the BCAT isoforms share 58% sequence homology and are structurally very similar, they possess distinct differences in catalytic efficiency and regulation (Davoodi *et al.*, 1998). These enzymes play significant roles in amino acid metabolism and whole-body nitrogen shuttling. The most ubiquitous isoform, $BCAT_m$, is found in mitochondria and is thought to be responsible for the majority of transamination outside the central nervous system with the highest levels of $BCAT_m$ recorded in the pancreas, kidney, stomach, and brain (Suryawan *et al.*, 1998). To date, the cytosolic isoform ($BCAT_c$) has only been isolated from brain, placenta, ovary, and the peripheral nervous system (Hutson, 1988; Hutson *et al.*, 1992, 1998; Hall *et al.*, 1993; Sweatt *et al.*, 2004a,b; Garcia-Espinosa *et al.*, 2007). Although these two isoforms predominate, two other spliced variants have been identified, namely, a novel alternatively spliced PP18b variant found in placental tissue, and a novel co-repressor for thyroid hormone nuclear receptors (P3) (Lin *et al.*, 2001; Than *et al.*, 2001). Both spliced variants are homologous to $BCAT_m$. Although the function of the PP18b variant is unknown, P3 acts as a co-repressor for thyroid hormone nuclear receptors. The biological significance of these variants remains to be determined.

Due to the ease with which the BCAA pass the blood–brain barrier and their role in glutamate metabolism, the subcellular localization of the BCAT proteins in brain has been extensively investigated (Oldendorf, 1973; Cremer *et al.*, 1976, 1979; Bixel *et al.*, 1997, 2001; Bixel and Hamprecht, 2000; Sweatt *et al.*, 2004a,b; Garcia-Espinosa *et al.*, 2007). In brief, $BCAT_c$ was only found in neuronal cells, localized to axons and nerve terminals in glutamatergic neurons, and concentrated in cell bodies in GABAergic neurons (Bixel *et al.*, 2001; Sweatt *et al*, 2004a,b). Conversely, $BCAT_m$ was the predominant isoform in astrocytic cells. However, low levels of $BCAT_c$ were detected in certain astrocyte populations using a cell culture model, but this was not reported in rat tissue (Bixel *et al.*, 1997, 2001; Bixel and Hamprecht, 2000). To date the BCAT proteins have not been mapped at the subcellular level in the human brain.

2.2.2.2 Cellular distribution of the ALT proteins

Early studies investigating the cellular distribution of ALT in rats suggested that there was only one cytosolic ALT isoform, with the mitochondrial form dismissed as cytosolic contamination of mitochondrial preparations. Subsequent reports have validated that there are two ALT isoforms (ALT1, cytosolic; and ALT2, mitochondrial), encoded by separate genes (ALT1–GTP gene and ALT2-GTP2 gene) (DeRosa and Swick, 1975; Sohocki *et al.*, 1997; Lindblom *et al.*, 2007; Glinghammar *et al.*, 2009). An alternatively spliced isoform of ALT2 (ALT2-2) has been suggested with a predicted sequence of 100 amino acids shorter than ALT2; however this isoform has not been characterized at the protein level and its function is unknown (Lindblom *et al.*, 2007). The ALT proteins play a pivotal role in mediating the passage of intermediates between gluconeogenesis and amino acid metabolism from the muscle to the liver, and more recently have been described as playing a role in the hypothetical alanine–lactate shuttle between neuronal and astrocytic cells (Waagepetersen *et al.*, 2000). Clinically, due to the high expression levels of ALT in liver it is considered to be one of the major biomarkers of liver dysfunction (see below).

Unlike BCAT, both ALT isoforms are widely expressed in rat and murine tissues.

Here high levels of ALT mRNA expression were reported in liver, muscle, and brown/white adipose tissue, with relatively lower ALT expression observed in rat colon, heart, and brain (Jadhao *et al.*, 2004; Yang *et al.*, 2009). With the exception of the kidney, these patterns of mRNA expression largely mapped to those observed in humans (Yang *et al.*, 2002a, 2009). While both ALT isoforms are highly expressed in rat adipose tissue, their expression in human adipose tissue has not been described. Although ALT brain activities have been reported in several rat models there are limited data on the pattern of ALT distribution in the human brain. A study described by Lindblom *et al* demonstrated that expression of ALT1 was below the detection limit for all brain samples analysed, whereas ALT2 showed low to moderate levels of expression in the cerebral cortex with intense staining in the cerebellum, highlighting the importance for further studies to validate ALT distribution in the brain (Lindblom *et al.*, 2007). These studies would extend our knowledge on the contribution of ALT proteins to glutamate metabolism (see below).

2.2.2.3 Cellular distribution of the AST proteins

Like ALT, the AST proteins are widely distributed with the highest expression found in striated muscle, myocardium, and liver tissues. With the exception of the red blood cells which only contain AST_c, all tissues have both isoforms, albeit at varying levels in different cell types. Because of their role in neuronal metabolism the distribution of these isoforms has been extensively studied in various brain preparations with particular focus on the mitochondrial isoform (AST_m). AST_m is not only targeted to mitochondria but also on the cell surface, confirming the role of AST_m as both a mitochondrial and plasma membrane protein (Cechetto *et al.*, 2002). Interestingly AST_m was found to be identical to a fatty acid-binding protein ($FABP_{pm}$), which has a role in permitting the uptake of long chain free fatty acid in cells (Stremmel *et al.*, 1990; Stump *et al.*, 1993; Bradbury and Berk, 2000). For full activity AST must first associate with binding proteins which transfer it to lipids on the inner mitochondrial membrane (Teller *et al.*, 1990). Functionally, AST proteins play a central role in glutamate metabolism and in the malate/aspartate shuttle which transfers reducing equivalents from the mitochondria to the cytosol in brain. Glutamate dehydrogenase (GDH), like AST, also catalyses the conversion of glutamate to α-ketoglutarate, albeit by a different mechanism (Leong and Clark, 1984). Whereas AST catalyses transamination (Box 2.1), GDH either adds an amino group to α-ketoglutarate utilizing NAD(P)H or removes an amino group from glutamate producing α-ketoglutarate with the reduction of NAD(P). Numerous studies have reported that both these enzymes are co-localized in cells and work in concert with each other to either drive the synthesis or degradation of amino acids (Lai *et al.*, 1977, 1986; Palaiologos *et al.*, 1988).

Immunohistochemical studies on rat brain showed differential staining between glutamatergic or GABAergic neurons. High expression of AST_c was reported in periglomerular cells of the olfactory bulb and basket cells, and in stellate cells of the cerebellum and second layer cells of the neocortex, whereas AST_m was found in mitral cells and glomerular regions of the olfactory bulb and golgi cells of the cerebellum (Kamisaki *et al.*, 1984). Similar reports of isoenzyme compartmentation were also described in the rat retina (Inagaki *et al.*, 1985, 1987). Although high levels of AST activity have been reported in neuronal cells, controversy surrounding the actual activity and indeed its contribution to glutamate metabolism in astrocytes exists, which is in part due to reports of the absence of the malate/asparate carrier (AGC) (see below). Further studies are required to determine the activity of AST relative to GDH in these brain preparations, their localization, and their specific roles in astrocytes.

2.3 The Role of Aminotransferases in Brain Metabolism

The compartmentalization of the aminotransferase isoforms in neuronal or astroglial cells and the observed differences in their catalytic

and regulatory mechanisms points to two functionally distinct proteins despite catalysing the same reaction. The aminotransferase proteins and their substrates play a significant role in normal brain function, driving several key metabolic pathways central to energy metabolism and neurotransmitter synthesis (Figs 2.4–2.6). A dysfunction of these pathways can potentially contribute to the pathogenesis of a number of neurodegenerative conditions such as Alzheimer's and Parkinson's disease (Choi, 1988; Esclaire *et al.*, 1997). A key metabolite linking these metabolic pathways is glutamate, the major excitatory neurotransmitter in the mammalian brain as well as the immediate precursor to GABA and glutathione (Attwell and Laughlin, 2001; Danbolt, 2001). Under normal physiological conditions glutamate plays a role in dendrite and synapse formation, but also plays a dominant role in glutamatergic transmission essential for memory and learning (Danbolt, 2001). Activation of excitatory neurotransmission mediated through an increase in calcium, signals the release of glutamate stores within the presynaptic neuron (Fig. 2.2). Excess glutamate not utilized by postsynaptic neurons is taken up by astrocytes, which express high levels of the glutamate-specific transporter (GLAST/EAAT1 and GLT1/EAAT2) facilitating the rapid and efficient removal of glutamate from the extracellular space. In fact transgenic mice that do not express GLT-1 are subject to glutamate toxicity (Tanaka *et al.*, 1997). Within the astrocyte, much of the glutamate is converted through

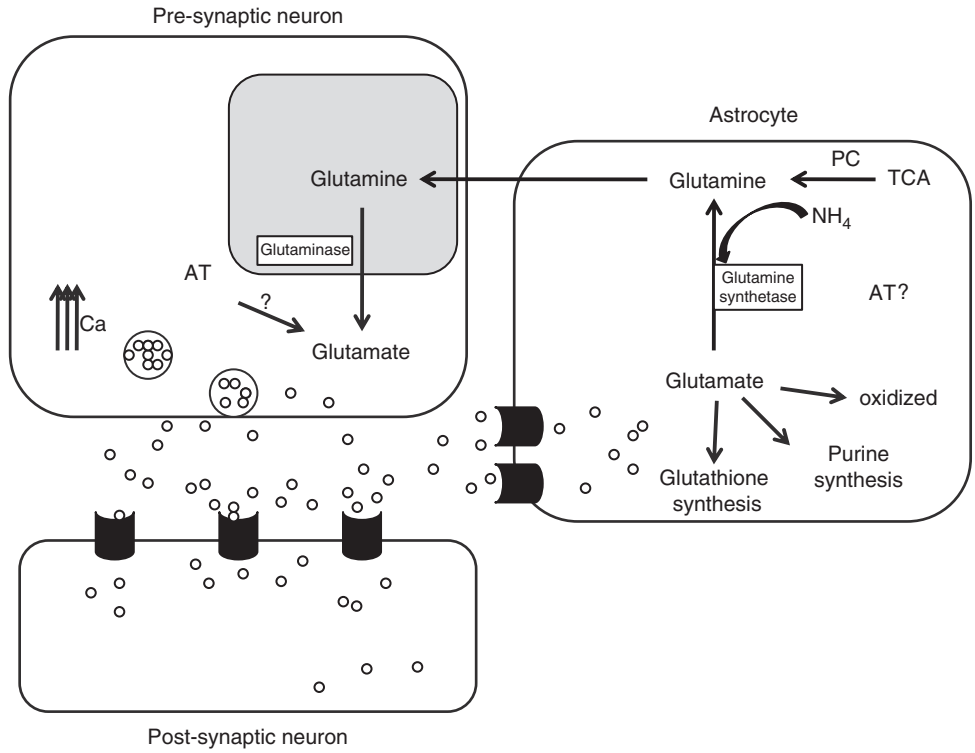

Fig. 2.2. The glutamate–glutamine cycle. Excess glutamate released from pre-synaptic neurons into the synaptic cleft is rapidly taken up by astrocytes through the specific glutamate receptors GLAST/EAA1 and GLT1/EAAT2. Within the astrocyte the majority of glutamate undergoes amidation to glutamine catalysed by glutamine synthetase. Non-neuroactive glutamine is released into the ECF for uptake by pre-synaptic neurons for regeneration of the neuronal glutamate pool. Glutamate may also be oxidized or utilized for the synthesis of glutathione or purines.

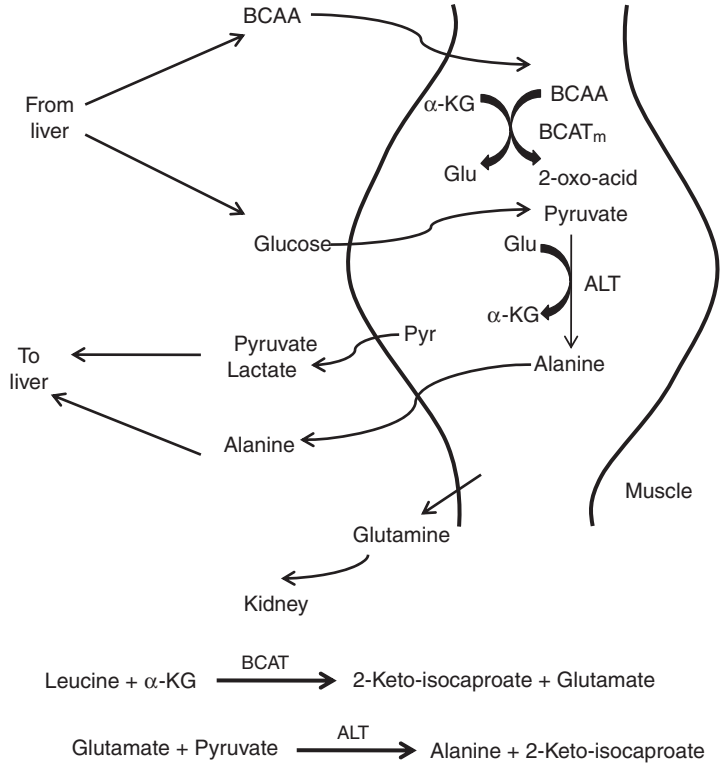

Fig. 2.3. Overview of the glucose–alanine cycle and BCAT in muscle. Both ALT and BCAT are highly concentrated in muscle, playing key roles in energy metabolism, particularly under nutritional stress. The BCAA which are not metabolized by liver are transported to muscle where they undergo transamination with α-keto glutarate. The BCAA, in particular leucine, is a major source for the amino group in amino acids such as alanine and glutamine. The alanine generated originates from pyruvate and therefore functions to recycle pyruvate originating from hepatic glucose. This cycle is termed the glucose–alanine cycle, which operates in parallel with the Cori cycle that also generates gluconeogenic substrates (Pyr/lactate) for the liver.

amidation to the non-neuroactive amino acid glutamine, by the microsomal enzyme glutamine synthetase (GS) that is restricted to astrocytes. Glutamine synthetase utilizes the steady supply of ammonia from blood (or brain metabolism) for glutamate synthesis which is then released into the ECF allowing for uptake by the pre-synaptic neuron to recycle the store of glutamate through deamidation of glutamine by mitochondrial phosphate-dependent glutaminase (Fig. 2.2) (Yudkoff *et al*. 1993, 1994). Thus, astrocytes and neurons play complementary roles in the glutamate–glutamine cycle, maintaining the neuronal glutamate pool at high concentrations, and preventing toxic elevations in the synaptic space. In astrocytes there are several fates for glutamate other than glutamine synthesis, dependent on substrate availability, the differential distribution and regulation of key metabolic enzymes (e.g. glutaminase, GDH, Malate dehydrogenase (MDH), AST, BCAT, ALT, Malic enzyme (ME), and GS), or whether the source of glutamate is exogenous or endogenous (Fig. 2.6). For example, when levels of external glutamate are low, the glial glutamate synthetase pathway is favoured, whereas in glutamate excess, considerable oxidation occurs (McKenna *et al*., 1996a,b).

Both glutamate and glutamine can be oxidized for energy in astrocytes and neuronal cells (McKenna *et al*., 1996a,b; Daikhin and Yudkoff, 2000). An estimated 30% of glutamate taken up by astrocytes is metabolized to

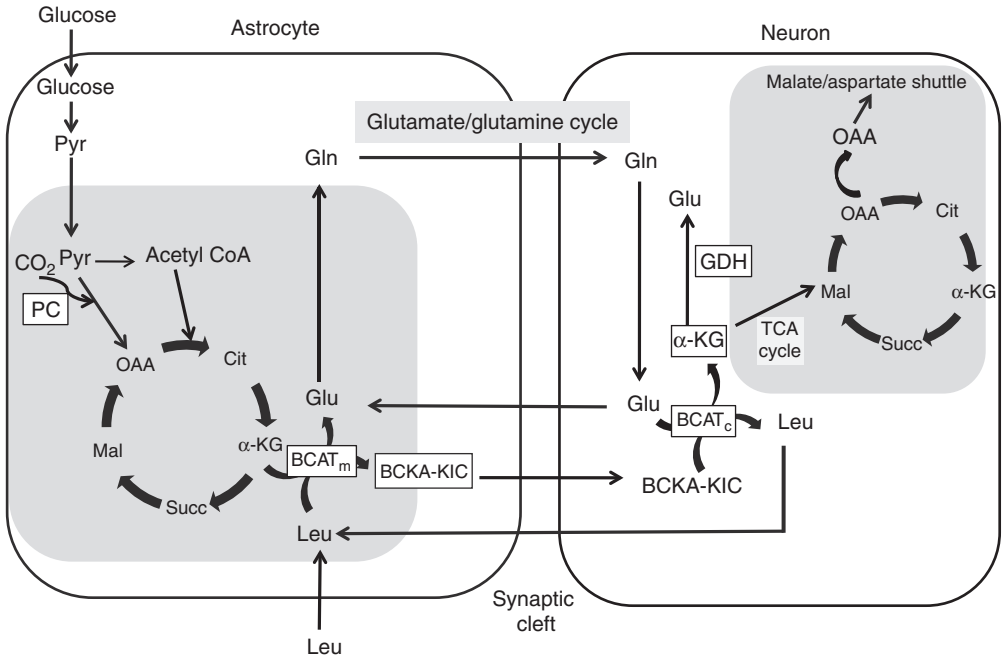

Fig. 2.4. The branched chain aminotransferase-branched chain keto acid shuttle. Leucine is actively taken up by astrocytes where it undergoes transamination via $BCAT_m$ forming glutamate and 2-ketoisocaproate (KIC). The glutamate formed can enter the glutamate–glutamine cycle whereas KIC, which is poorly metabolised by astrocytes, is further metabolized in neuron cells. The keto acid is subsequently transaminated with glutamate to regenerate leucine, which can be subsequently returned to the astrocyte to complete the cycle. These cycles also operate with the TCA cycle and GDH metabolism (Yudkoff *et al.*, 1990; Hutson *et al.*, 2001).

lactate involving TCA cycle intermediates and the pyruvate/malate cycle (Sonnewald *et al.*, 1993; Gamberino *et al.*, 1997). Glutamate in astrocytes is also used in the production of purines and key metabolic amino acids such as glutathione (Fig. 2.2) (Shank and Aprison, 1981; Yudkoff *et al.*, 1988). Therefore, anaplerotic pathways must interface with the glutamate–glutamine cycle to regenerate this 'lost' glutamate necessary to sustain efficient neurotransmission. Pyruvate carboxylase, an enzyme found solely in astrocytes, utilizes brain CO_2 to replenish the carbon required for the TCA cycle, which as a result contributes to the overall concentration of glutamine produced (Oz *et al.*, 2004). A limiting factor in this reaction is the source of nitrogen, where the BCAA, aspartate and more recently alanine serve as potential nitrogen donors (Shank *et al.*, 1985; Bixel and Hamprecht, 1995; Yudkoff *et al.*, 1996a,b; Yudkoff 1997; Hutson *et al.*, 1998, 2001; Kanamori *et al.*, 1998; Lieth *et al.*, 2001; Magistretti, 2009). The respective roles of the aminotransferase proteins are discussed in the following sections.

2.3.1 The role of BCAT in brain metabolism

The BCAA easily traverse the blood brain barrier, with leucine more readily accepted than other amino acids (Oldendorf, 1973; Smith *et al.*, 1987). Conversely, glutamate and glutamine are poorly taken up, highlighting the importance of glutamate synthesis in the brain. It has long been established that in peripheral tissues the role of the BCAA, in particular leucine, is to act as a major nitrogen donor for glutamate and glutamine synthesis (Fig. 2.3) (Goldberg and Chang, 1978). Skeletal muscle harbours high concentrations of $BCAT_m$, mediating the formation of glutamate

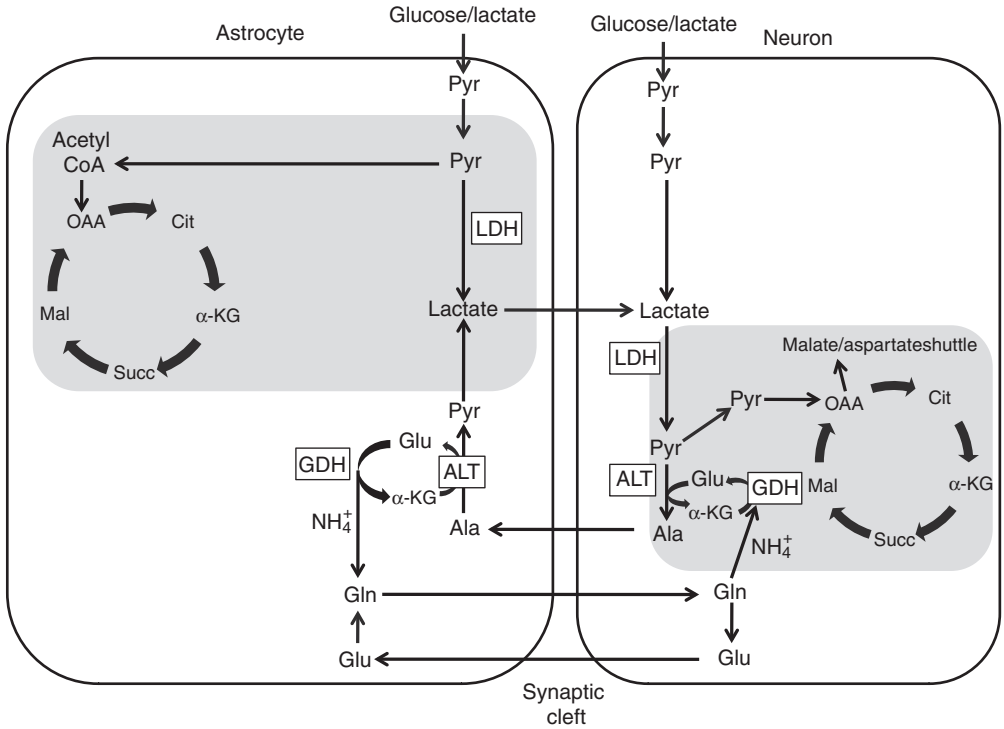

Fig. 2.5. The alanine–lactate shuttle operates with the glutamate–glutamine cycle. Lactate produced in astrocytes can be exchanged in part with alanine from neuronal cells. Lactate is subsequently metabolized to pyruvate which has two fates: i) The TCA cycle, or ii) transamination with glutamate via ALT. The alanine formed through transamination is thought to carry the amino group through GDH metabolism from glutamine deamination to glutamate. The alanine produced acts as a nitrogen donor in astrocytes, ultimately used to regenerate glutamate (adapted from Waagepetersen *et al*., 2000).

and α-keto glutarate from BCAA exported from the liver (Fig. 2.2). The role of leucine as a nitrogen donor in brain metabolism has since been extensively studied, where the BCAT proteins are considered to play a key role in the oxidation and synthesis of glutamate owing to both their high expression and subcellular localization.

In rat brain slices the BCAA are metabolized faster than they are incorporated into proteins, supporting the theory that the BCAA serve functions other than just an energy source (Chaplin *et al*., 1976). In particular, leucine is readily metabolized in astrocytes, where uptake of leucine by glial cells is mediated by a sodium-independent process (Brookes, 1992, 1993). A combination of kinetic and metabolic studies in astrocytes, measuring the incorporation of [^{15}N]leucine into glutamate and glutamine, suggested that the rate of transamination of leucine is greatly favoured over complete oxidation with approximately 30% of the nitrogen of glutamate/glutamine derived from leucine alone (Brand, 1981; Brand and Hauschildt, 1984; Harper and Benjamin, 1984; Yudkoff *et al*., 1990). These studies, among others, support the hypothesis that although transamination is completely reversible it seems that BCAT transamination in the direction of glutamate and α-keto acid formation is favoured in astrocytes, whereas the reverse holds true for neuronal cells. These produce leucine and α-keto glutarate, thought to complement the glutamate–glutamine cycle (Yudkoff *et al*., 1996b; Yudkoff 1997; Daikhin and Yudkoff, 2000). Both *in vivo* rat brain and *ex vivo* rat retina models (accepted models of glutamatergic

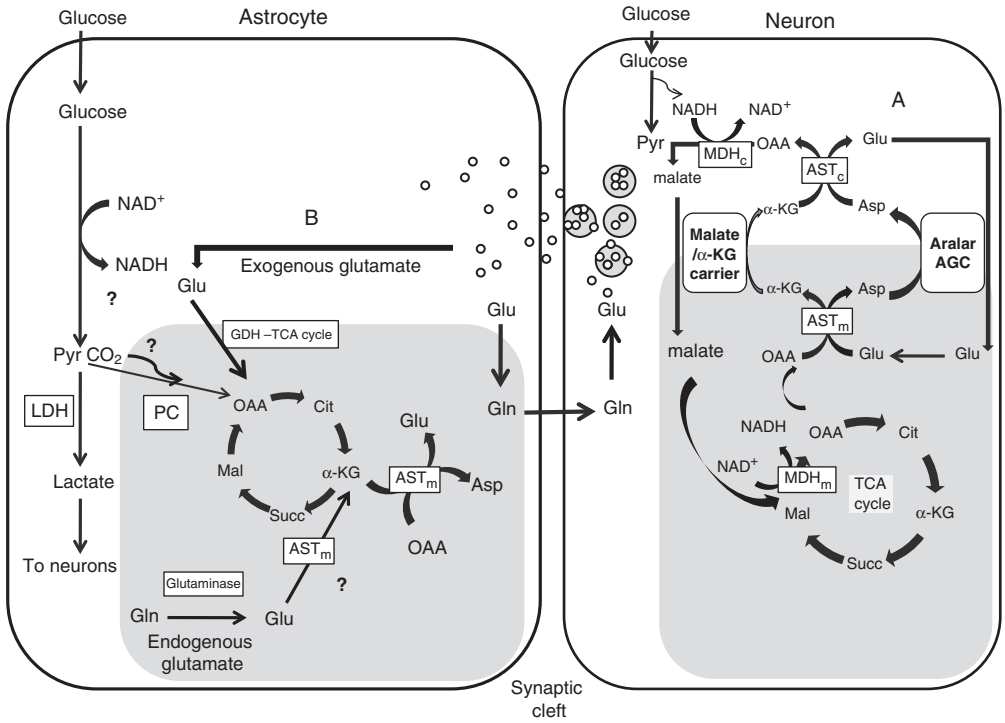

Fig. 2.6. Aspartate aminotransferase proteins in the brain. A) In neuronal cells AST_m and AST_c operate with the malate/α-KG carrier, the aralar AGC carrier, malate dehydrogenase (MDH), and glutaraldehyde dehydrogenase (GDH), which operate in metabolic complexes to channel NADH-reducing equivalents to the mitochondria for further oxidation. B) The role of AST_m is thought to work in synergy with GDH in the metabolism of glutamate. Here, exogenous glutamate is metabolized by GDH, whereas AST_m is thought to process glutamate produced endogenously.

neurons) using gabapentin as an inhibitor of $BCAT_c$ have also supported the *de novo* synthesis of glutamate (LaNoue *et al*., 2001; Lieth *et al*., 2001). These studies confirmed that gabapentin inhibited the *de novo* synthesis of glutamate by 30% in retina cells and up to 50–60% of added leucine transamination.

Not only is synthesis affected but so too is oxidation. Both retinal Muller cell (retinal astroglia) and cultured astrocyte models confirmed that transamination was a necessary prerequisite step to glutamate oxidation, where oxidation was blocked by the transaminase inhibitor aminooxyacetic acid (AOAA) (McKenna *et al*., 1996a,b; Lieth *et al*., 2001). To fit with the proposed model these results would imply that the supply of branched chain keto-acids (BCKA) would affect the extent of glutamate oxidation. In cultured rat astrocytes, oxidation of [^{14}U]glutamate was stimulated by addition of BCKA (Hutson *et al*., 1998). In retinal cells the addition of BCKA, gabapentin, and BCKA and gabapentin together, resulted in the stimulation of glutamate oxidation with the latter showing the greatest degree of stimulation. These findings, together with the subcellular localization of $BCAT_m$ to astrocytes and $BCAT_c$ to neuronal cells, led to the development of the BCAA–BCKA shuttle hypothesis between the astrocyte and neuron, which works together with the glutamate/glutamine cycle (Fig. 2.4) (Yudkoff *et al*., 1996a; Yudkoff, 1997; Hutson *et al*., 1998, 2001). Here, mitochondrial BCAT catalyses the transamination of the BCAA in astrocytes. The resulting BCKA, which are poorly metabolized by astrocytes, are shuttled to neuronal cells for further metabolism,

while glutamate enters the glutamate/glutamine cycle. $BCAT_c$, which is neuronal specific, catalyses transamination of the BCKA with glutamate back to the BCAA, which exit the neuron and return to the astrocyte. α-ketoglutarate may also undergo reductive amination to glutamate via neuronal GDH but is thought not to be a serious contender, as the flux in this direction is low (Yudkoff *et al*., 1990; Hutson *et al*., 2001). Therefore, BCAT proteins in brain metabolism do not only facilitate energy metabolism, but also provide essential nitrogen for the anaplerotic regeneration of glutamate.

2.4 Alanine Aminotransferases and Glutamate

In muscle, BCAA and alanine metabolism are intrinsically linked with glutamate metabolism. During gluconeogenesis both alanine and glutamine are the predominant amino acids which leave the muscle, with alanine preferentially taken up by the liver (Fig. 2.3). Evidently ALT plays a more dominant role in protein metabolism of liver and muscle relative to brain, as the levels of ALT are significantly higher. Alanine released from muscle is derived from pyruvate and glutamate, releasing α-keto glutarate thought to drive the TCA cycle particularly during exercise; this is known as the glucose alanine cycle (Fig. 2.3) (Rutten *et al*., 2005). This is closely linked to the glucose/lactate cycle (Cori cycle), which operates in parallel during gluconeogenesis. Thus, carbohydrate and protein metabolism operate cooperatively, where the direction of metabolism is substrate driven. The role of ALT in brain metabolism has been described in the hypothetical lactate–alanine shuttle between brain cells, with its metabolites potentially linked with glutamate metabolism (Fig. 2.5) (Pellerin and Magistretti, 1994; Peng *et al*., 1994; Waagepetersen *et al*., 2000; Schousboe and Waagpetersen, 2005; Bak *et al*., 2006).

Glucose is considered the main energy source of the brain, where both neuronal and astrocytic cells are capable of its metabolism. Relative to other metabolites glucose is readily taken up by the brain and is available in high concentrations in the blood. Conversely, lactate cannot readily pass the blood–brain barrier (Cremer *et al*., 1976, 1979) and its levels in the blood fall short in maintaining the energy requirements of the brain, even though neuronal cells express both lactate and glucose transporters (Bergersen *et al*., 2002). It has been demonstrated that astrocytes have greater glycolytic activity than neuronal cells, with the lactate produced transported to neuronal cells for further oxidation (Schousboe *et al*., 1997; Waagepetersen *et al*., 2000; Pellerin, 2003). The lack of metabolic machinery (i.e. the malate/aspartate shuttle) in astrocytes, which would otherwise drive glycolysis through to complete oxidation in the mitochondria, support these findings. In fact, with the proposed lactate–alanine shuttle it was suggested that neuronal cells may actually metabolize lactate as efficiently as glucose, a subject actively debated in the literature (Schurr *et al*., 1997 a,b,c; Cater *et al*., 2001; Waagepetersen *et al*., 2000; Bak *et al*., 2006, 2007, 2009). Metabolic studies utilizing [U^{13}C]lactate incubated with glutamatergic cerebellar granule cells, astrocytes, and co-cultures demonstrated that alanine was preferentially formed or released into the medium in neuronal cells. Incubation of these cells with [U^{13}C]alanine resulted in the preferential enrichment of alanine in astrocytes relative to neuronal cells, suggesting that neurons preferentially synthesize and release alanine, and astrocytes favour uptake supporting a different functional role of alanine metabolism in brain cells. Thus, as described for BCAA in the BCAA–BCKA shuttle, the role of alanine as a carrier for nitrogen between neurons and astrocytes in exchange for lactate was suggested (Fig. 2.5). In this role it was proposed that alanine would serve to transport excess ammonia from neuronal cells to astrocytes, generated from the metabolism of glutamine to glutamate by mitochondrial glutaminase, where it could be utilized by GS for glutamine synthesis from glutamate (Waagepetersen *et al*., 2000). To establish a link between the alanine–lactate cycle and the glutamate–glutamine cycle, neuronal and astrocyte cells were incubated with [5-^{15}N] glutamine and [^{15}N]alanine, respectively. A minor 3.3% enrichment of alanine in neuronal cells and 22% monolabelling in glutamate and glutamine in astrocytes was reported, offering

some evidence in support of the shuttle; however, the actual extent to which it contributes as an anaplerotic pathway is still in dispute.

Production of lactate in astrocytes is thought to correlate with an increase in glycolysis stimulated through glutamate uptake (Pellerin *et al*., 1998). In brief, it was proposed that lactate generated from astrocytes or through uptake from the peripheral blood is converted to pyruvate by lactate dehydrogenase (LDH), which is further utilized for energy production through the TCA cycle or as a substrate for ALT (Fig. 2.5). In neuronal cells ALT catalyses the transamination of pyruvate to alanine which results in the consumption of glutamate and the generation of a-ketoglutarate. Alanine that is produced is released and taken up by astrocytes to undergo further transamination to pyruvate and α-keto glutarate, completing the cycle (Fig. 2.5) (Waagepetersen *et al*., 2000). Here, the role of alanine was proposed to act as a nitrogen carrier from neuronal cells to astrocytes. It was suggested that alanine formed as a result of GDH activity utilizes the excess ammonia formed from the glutaminase reaction, and channels the much-needed source of nitrogen into astrocytes. In retinal Muller cells 60% of glutamate synthesis occurred via AOAA-sensitive transamination (LaNoue *et al*., 2001). Furthermore, using the inhibitor L-cycloserine, *de novo* synthesis of glutamate was inhibited to a greater degree relative to the specific inhibition of BCAT using gabapentin. This suggests that this alanine shuttle is potentially active in intact neural tissue and that it provides a proportion of nitrogen for glutamate synthesis in the retina (LaNoue *et al*., 2001). Evidently, ALT does play a role in brain glutamate metabolism; however, the extent to which this contributes to glutamate levels should be considered in the future with the distribution/activity of ALT in neuronal and glial cells.

2.5 Aspartate Aminotransferases and their Role in the Malate/Aspartate Shuttle and Glutamate Metabolism

Functionally, AST proteins play a central role in the malate/aspartate shuttle and in glutamate metabolism. In neuronal cells, the synaptic terminals which are rich in mitochondria have high malate/asparate shuttle activity (Cheeseman and Clark, 1988) and high levels of AST and GDH activity (McKenna *et al*., 2000a,b), whereas the activity of AST in astrocytes is reported to be considerably lower. Oxidation of glucose to pyruvate yields NADH, a major reducing equivalent which drives the TCA cycle to yield the maximum energy from glucose, which is essential for brain function. The function of the malate/aspartate shuttle is to avoid the build-up of the ratio of $NADH/NAD^+$, which serves to favour metabolism moving in the direction of lactate production, rather than oxidation of pyruvate in the TCA cycle, which would decrease the energy output from glycolysis. Although other shuttle mechanisms may operate, the two most studied shuttles include the malate–aspartate shuttle and the glycerol 3–phosphate shuttle, with the malate–aspartate shuttle considered to be particularly important in neurons and the most important in brain (Fig. 2.6A) (Cheeseman and Clark, 1988). The role of the glycerol 3–phosphate shuttle is disputed, due to conflicting reports of the subcellular localization of this shuttle in various brain cells (Nguyen *et al*., 2003).

The AST isoforms are central to the operation of this shuttle as they are the rate-limiting enzymes; evidence suggests that this shuttle is more active in neuronal cells relative to astrocytes (McKenna *et al*., 2006). The AST enzymes work with several other proteins including the aspartate/glutamate carrier (AGC – aralar 1 isoform), the malate/α-ketoglutarate carrier, and GDH and MDH, which have been shown to form metabolomic complexes through physical associations with each other (Ramos *et al*., 2003; McKenna *et al*., 2006). These physical interactions between enzymes are thought to facilitate the transfer of substrates between enzymes thus maximizing catalysis. The *in vivo* importance of these interactions is currently unknown. The mitochondrial enzyme AST_m operates in the direction of aspartate synthesis, and the cytosolic in the direction of aspartate conversion to oxaloacetate (OAA). Briefly, the mitochondrial membrane is impermeable to NADH formed during the glycolytic cycle. To facilitate the transfer of reducing equivalents, OAA is reduced to malate in the cytosol, which is exchanged for

α-keto glutarate across the mitochondrial membrane. Malate dehydrogenase catalyses the release of the reducing equivalents from malate with the formation of OAA. Here, mitochondrial AST catalyses the transamination of OAA and glutamate to aspartate and α-keto glutarate, respectively. Both aspartate and α-keto glutarate are subsequently transferred to the cytosol where its cytosolic counterpart, AST_c, regenerates glutamate and OAA from cytosolic aspartate and α-ketoglutarate (Fig. 2.6A). Over the last few years several groups using immunohistochemistry and *in situ* hybridisation have demonstrated that astrocytes have either no expression or very low levels of AGC, one of the key components of the malate/aspartate shuttle (Ramos *et al*., 2003; Xu *et al*., 2007), which suggests that the direction of metabolism results in the production of lactate that must be exported to neuronal cells for further oxidation (Fig. 2.6) (McKenna, 2007; Xu *et al*., 2007).

In addition to their role in the transfer of reducing equivalents, the AST proteins have been shown to play an important part in glutamate metabolism (Safer and Williamson, 1972; Scholz *et al*., 1998; Chatziioannou *et al*., 2003). The rate of oxidation of glutamate differs in neuronal and astrocytic cells. In astrocytes glutamate is oxidized at a rate almost twice that of glutamine, which supports the role of astrocytes in disposing excess glutamate. It has been reported that the role of AST_m in glutamate metabolism is dependent on the origin of glutamate. Using ^{13}C NMR tracer experiments and inhibitors of aminotransferase proteins such as AOA, it was proposed that oxidation of exogenous glutamate is primarily through the action of mitochondrial GDH, whereas glutamate synthesized endogenously from glutamine (oxidized for energy by glutaminase) is metabolized by AST (Fig. 2.6B) (McKenna *et al*., 1993, 1996b). In fact, a congenital deficiency or mutation of GDH can result in neurological disturbances (Plaitakis *et al*., 1982; Kelly and Stanley, 2001). Irrespective of this, metabolism by either GDH or AST results in the production of key metabolites which enter into the TCA cycle (Sonnewald *et al*., 1993; McKenna *et al*., 1996b) and are thought to be particularly important in the disposal of excess glutamate. ^{13}C-NMR studies have shown that the carbon skeleton from [U-^{13}C]glutamate can be traced to lactate, aspartate, or resynthesized glutamate from the TCA cycle (Sonnewald *et al*., 1993; McKenna *et al*., 1996a).

As described for astrocytes, both glutamate and glutamine can be oxidized to CO_2 in neuronal cells (McKenna *et al*., 1993, 1996a); however, here glutamine is metabolized preferentially at a rate five times faster than glutamate. Interestingly, endogenous glutamate is oxidatively deaminated by GDH, which appears to be the opposite to that observed in astrocytes (Tildon *et al*., 1985; McKenna *et al*., 1993, 1996a). As both AST and GDH have high activity in the mitochondrial synaptic regions it suggests that the relative importance of these two enzymes in the oxidation of glutamate is substrate driven (Erecinska *et al*., 1990; McKenna *et al*., 2000b). Although transamination by AST is freely reversible, the direction or flow of metabolism will be dictated by the concentration of metabolites and possibly how they can influence the metabolomic interactions of AST with other membrane-bound proteins such as glutaminase (Teller *et al*., 1990; Cooper, 2001). Furthermore, when energy substrates are sufficient, α-keto glutarate formed in the TCA cycle can be metabolized to glutamate through transamination (Sonnewald *et al*., 1993). Therefore, taken together these studies suggest both AST and GDH play a central role in glutamate metabolism, favouring catabolism in astrocytes and conservation in neuronal cells.

2.6 Pathological Conditions Resulting from Impaired Aminotransferase Metabolism

To date there are no known mutations of the BCAT, AST, or ALT aminotransferase proteins contributing to a pathological condition. However, a build-up of their substrates or products can lead to disorders leading to neurodegeneration (e.g. glutamate toxicity in Alzheimer's disease). The most accepted consequence of altered BCAA metabolism is a mutation of the BCKDH complex resulting in the accumulation of the BCAA and α-keto acids leading to neuronal dysfunction (see below). Although not discussed in detail in

this chapter, the only aminotransferase protein with a known mutation is the alanine:glyoxylate aminotransferase (AGT, EC 2.6.1.44), a PLP-dependent metabolic enzyme which catalyses the transamination of alanine and glyoxylate to pyruvate and glycine. This enzyme is characteristically found in the peroxisomes distributed largely in hepatocytes. In humans, AGT is encoded by the AGXT gene, mutations of which give rise to dysfunctional proteins resulting in the overproduction of oxalate (Coulter-Mackie and Rumsby, 2004; Danpure, 2006). Excess oxalate leads to the progressive accumulation of insoluble calcium oxalate in the kidney and urinary tract leading to urolithiasis, often accompanied by systemic oxalosis, which ultimately results in renal failure.

2.6.1 Maple syrup urine disease

Maple syrup urine disease is a congenital disease characterized by a build-up of both BCAA and their respective keto-acid derivatives. These metabolites are neurotoxic to cells in the cerebrospinal fluid, blood, and tissues resulting in the patient presenting with symptoms such as neurological dysfunction, seizures, and infant death (Silberman *et al*., 1961; Chuang, 1998, 2006). MSUD is an autosomal recessive disorder caused by a deficiency of the multienzyme complex, the branched-chain α-keto acid dehydrogenase complex (BCKDH), the rate-limiting step of transamination (Dancis *et al*., 1959, 1960; Menkes, 1959, 1962; Dankis 1964). This complex is composed of three catalytic subunits, the E1, E2, and E3 subunits (Harris *et al*., 2004), where all three units are essential for enzymatic activity (Danner and Doering, 1998). At least 150 mutations in the BCKDH complex genes have been reported, with the most disease-causing mutations seen in E2 (Chuang, 1998; Danner and Doering, 1998, Chuang and Chuang, 2000; Chuang *et al*., 2006). Five classifications of MSUD have been identified (varying from severe classic forms to mild variant types, and also with a thiamine- responsive form), based on the residual BCKDH activity, the age of onset, and the concentration of leucine in serum (Duran and Wadman, 1985; Chuang and Chuang, 2000; Chuang *et al*., 2006).

Although treatment through restriction of the BCAA in the diet has most value in milder forms of the condition, patients that are not compliant with their diet or those with more severe forms of the disease are still subject to many side effects (Snyderman, 1986, 1988). Investigation into alternative therapies using animal models has been described (Klivenyi *et al*., 2004; Wu *et al*., 2004; Homanics *et al*., 2006). Briefly, the usefulness of these models varies widely, from creating homozygous lethal knockouts, to knockouts that phenotypically resemble BCKDH but on further investigation revealed a $BCAT_m$ mutation (Wu *et al*., 2004). More useful models include a classical MSUD and intermediate MSUD design (Homanics *et al*., 2006; Zinnanti *et al*., 2009). In the homozygous mouse, relative to wild-type (WT), levels of BCAA to alanine were 22-fold higher and 16-fold higher for the cMSUD and iMSUD, respectively. In contrast to the increase in BCAA levels, alanine, glutamate, and glutamine in the blood were all significantly reduced in iMSUD mice compared with WT, with low levels of aspartate and gamma-aminobutyric acid also uniformly reduced. The reduced levels of neurotransmitters are likely to cause encephalopathies such as coma. These models will provide a wealth of knowledge for pathological analysis and metabolic profiling of blood, brain, and other tissues (Zinnanti *et al*., 2009).

2.6.2 Glutamate toxicity and neurodegeneration

Under normal physiological conditions glutamate plays a role in dendrite and synapse formation, and also a dominant role in glutamatergic transmission, essential for memory and learning (Danbolt, 2001). However, high levels of glutamate result in this amino acid becoming a potent neurotoxin. This has been reported to lead to increased expression of tau protein, neuronal degeneration, and cell death (Choi, 1988, 1990; Esclaire *et al*., 1997). The effect of the excessive synaptic release of glutamate is largely mediated by an increase in the entry of calcium into neurons (Kaplan and Miller, 1997; Sattler and Tymianski, 2000; Mattson, 2003, 2007, 2008), which is the predominant secondary messenger

for neurotransmitters and neurotrophins (Choi, 1988; Kaplan and Miller, 1997). An overload of calcium, observed in the brain tissue of patients with AD has been shown to evoke acute degenerative conditions (Mattson and Chan, 2003; Mattson, 2007). Thus, a strong relationship exists between excessive calcium influx and glutamate-triggered neuronal injury. Recently published studies using targeted proteomics in neuronal cells have shown that BCAT proteins have redox-mediated associations with several neuronal proteins involved in G-protein cell signalling, indicating a novel role for BCAT in cellular redox control (Conway *et al.*, 2008). Interestingly, the brain-derived neurotrophin factor, which mediates its action through calcium cell signalling, causes up-regulation of BCAT (Numakawa *et al.*, 2002, 2009; Madeddu *et al.*, 2004). These findings indicate that the BCAT proteins may have fundamental links with calcium-mediated signalling, and because of its primary role in producing glutamate, understanding this mechanism may enhance our knowledge of how glutamate can reach toxic levels in neurodegenerative diseases such as AD, offering potential sites for targeted therapy.

2.6.3 Redox sensitivity of BCAT

Generation of reactive nitrogen and oxygen species (RNS and ROS, respectively) can occur through calcium overload, which as previously mentioned can be generated through glutamate neurotoxicity (Kaplan and Miller, 1997; Sattler and Tymianski, 2000; Mattson, 2003, 2007, 2008). Mitochondrial dysfunction can also generate reactive species, in particular peroxynitrite. Targets of these harmful species include reactive thiols of receptive proteins, resulting in changes to the structure and/or function of a protein, ultimately leading to metabolic imbalances resulting in cell death. The BCAT proteins are unique among the mammalian aminotransferases in that they contain a redox-active CXXC motif subject to reversible mofiication by both ROS and RNS, potentially serving as a biological control point (Conway *et al.*, 2002, 2003, 2004, 2008; Coles *et al.*, 2009; Hutson *et al.*, 2009). Response to cellular stress varies between isoforms, with $BCAT_m$ being completely inactivated by both ROS and RNS, whereas $BCAT_c$ is only partially sensitive to air oxidation and the nitric oxide donor, S-nitrosoglutathione (GSNO). Low concentrations of GSNO caused a reversible time-dependent loss in 50% of $BCAT_c$ activity, characterized predominantly through S-nitrosation (a reaction transferring a NO group to the reactive cysteine of this protein) (Coles *et al.*, 2009). However, increased exposure to GSNO resulted in a shift towards S-glutathionylation (addition of GSH to the reactive thiol), a marker of oxidative stress (Coles *et al.*, 2009). Recent studies have demonstrated that both S-nitrosation and S-glutathionylation of proteins occur in the brains from patients suffering with AD, which is directly correlated to the misfolding of proteins (Yao *et al.*, 2004; Benhar *et al.*, 2006; Uehara *et al.*, 2006; Fang *et al.*, 2007; Lipton *et al.*, 2007; Nakamura and Lipton, 2007, 2008, 2009; Cho *et al.*, 2009). Investigation as to how these mechanisms of S-nitrosation or S-glutathionylation regulate the hBCAT proteins *in vivo*, relative to glutamate toxicity and protein misfolding, may contribute to the understanding of these fundamental pathways involved in the pathogenesis of AD.

2.7 Aminotransferase Proteins as Biomarkers of Disease

Not only do the aminotransferase (AST and ALT) proteins have significant roles in whole-body nitrogen metabolism: they have also been used for decades as biomarkers of disease, most notably liver disease (Panteghini, 1990). The chapter will therefore digress to accommodate their role in clinical biochemistry, detailing how their measurements in serum can assist in the differential diagnosis of hepatic conditions. Biomarkers are used in screening, diagnostics, prognostics, or monitoring of patient outcome. The role of AST and ALT as biomarkers is primarily diagnostic with some prognostic applications. Ideally a diagnostic biomarker should be differentially specific, released in a timely fashion, with rapid robust validated methods of analysis. As both AST and ALT are found in several tissues they fall short as 'ideal' markers of disease. For example, erythrocyte levels of

AST are 10–15 times greater than that measured in serum, so mild elevations could suggest haemolysis rather than hepatic injury. However, to increase their clinical utility and diagnostic relevance, AST and ALT are grouped with other tests of liver function traditionally known as 'liver function tests' (LFT) (Table 2.1). It is relevant to note that although these tests are generically associated as LFT, they do not reflect liver function but rather the structural integrity of liver cells. True liver function tests would include the measurement of albumin, total protein, bilirubin, and/or prothrombin time. As ALT is localized only in the cytosol of liver, and AST is found in both mitochondria and cytosol, the ratio of AST/ALT can be used as an index of the severity of hepatic damage and indeed as a good prognostic indicator. Should the level of AST exceed ALT, damage to the cell is extensive and prognosis is poor.

Abnormal levels of the aminotransferase proteins may reflect both acute and chronic conditions and are interpreted in conjunction with the clinical and biochemical presentation of the patient. Patient history is of utmost importance in this differential diagnosis, as medications (including herbal remedies and over-the-counter preparations), co-morbid conditions, risk factors for viral hepatitis, and age can considerably influence the diagnosis. The reference limits for the aminotransferase proteins can vary among laboratories; however, examples of reference ranges used are summarized in Table 2.1. The use of AST and ALT as biomarkers is normally expressed with respect to the level of magnitude above the reference range, and more so the pattern of alteration often with respect to other LFT markers (Table 2.1). Levels can be defined as mild (≤2X), moderate (3–5X), or a marked increase (≥10–100X) above the upper reference limit (URL), where the rate and nature of change also illustrates the extent of cellular damage. It is important to note that these classifications are broad and may differ among clinicians. Therefore, patient history, the relative degree or elevation, in addition to the pattern of increase of the aminotransferase proteins relative to the other LFT, can differentiate between the causes of both acute and chronic liver disease.

2.7.1 Mild elevation of ALT and AST

The most common causes for a mild elevation of ALT and AST include acute alcoholic-induced or non-alcoholic fatty liver disease (NAFLD), where the activities of AST/ALT are reported as ≤2X the URL (Bayard *et al.*, 2006). Generally, levels of ALT exceed that of AST in acute liver damage, with the exception of toxin-induced or alcoholic hepatitis and Reyes syndrome (Dufour *et al.*, 2000a,b). One explanation for this change in the ratio is due to a deficiency in PLP, common in alcoholics. This has an impact in two ways: ALT is more sensitive to this loss than AST, and alcohol induces the release of AST_m, thus increasing the total amount of serum AST (Dufour *et al.*, 2000a,b). Mildly elevated levels of AST and ALT are also reported for chronic conditions associated with hepatitis B and C, and cirrhosis. A ratio of AST/ALT greater than 1.0 is suggestive of advanced liver disease with a greater risk of advanced fibrosis indicative of a poor prognosis (Williams *et al.*, 1988; Giannini *et al.*, 1999, 2003). An AST/ALT ratio ≥1 can be found in 4% of patients with chronic hepatitis C and in 79% of patients who have cirrhosis (Williams *et al.*, 1988; Giannini *et al.*, 1999, 2003). If these conditions are excluded, consideration for more rare causes of mildly elevated AST and ALT must be evaluated, such as haemochromatosis, Wilson's disease (in younger patients), autoimmune liver disease, and α-1-antitrypsin deficiency (Krawitt, 1996; Morrison and Kowdley, 2000; Ferenci *et al.*, 2005). Patients with autoimmune disease, based on elevated levels of hypergammaglobulinaemia, also present with low levels of aminotransferase activity.

Pathologically the first stage of alcoholic liver disease is the appearance of large fatty deposits, without evidence of clinical or biochemical abnormalities. Currently, no biomarker can detect early stage hepatic injury. In stage two, alcoholic hepatitis, increased levels of AST and ALT may be mild or moderate. Because other conditions can also give rise to mild elevations of AST and ALT, γ-glutamyl transferase, a toxin-inducible enzyme often elevated in both alcoholic and drug induced injury and alkaline phosphatase (ALP), may be assessed and taken together with the

Table 2.1. Serum levels of AST and ALT relative to other markers of hepatic injury.

Biomarker	Cellular distribution	Predominant tissue localization	Reference range	Cholestasis	Hepatic ischaemia or toxic injury	Acute viral hepatitis	Acute alcoholic liver disease	Chronic liver disease	Hepatic cirrhosis
Biomarkers of structural integrity									
ALT	Cytoplasm[a]	Liver, muscle and kidney	5–50 IU l^{-1}	Mild or moderate	↑↑↑	↑↑	Mild or moderate	Normal or ↑	Normal or ↑
AST	Cytoplasm and mitochondria	Liver, heart, skeletal muscle, brain, and kidney	AGE RANGE ↑ 5 wks (6–122 IU l^{-1}), 6 wks–1 yr (6–71 IU l^{-1}) 1–4 yrs (6–51 IU l^{-1}) ≥5 yrs (6–35 IU l^{-1})	Mild or moderate	↑↑↑	↑↑	Mild or moderate	Normal or ↑	Normal or ↑
γ Glutamyl transferase	Cell membrane	Liver, bilary canals, kidney	≤ 55 IU l^{-1}	Mild or moderate	↑↑	↑↑	↑↑	↑↑	↑↑
ALP	Cell membrane	Liver, bone, and kidney	≤ 150 IU l^{-1}	Moderate or marked	↑	↑↑	↑↑	↑↑	↑↑
Functional biomarkers									
Bilirubin		Liver (but also extra hepatic)	≤ 20 μmol l^{-1}	Mild or moderate	Normal or mild	Dependent on the type of infection[b]	Mild or moderate	↑↑	↑↑↑
Albumin[c]		Liver	35–45 g l^{-1}	Normal	Normal	Normal	Normal or ↓	↓	↓
Prothrombin time[d]		Liver	≤ 150 s	Normal	Mild or moderate	Normal or ↑	Normal or ↑	↑↑	↑↑

[a]Only cytosolic form present in the liver; other tissues have both a cytosolic and mitochondrial isoform.
[b]Levels of bilirubin will be elevated relative to infection with hepatitis A, B, C, etc., and the extent of damage.
[c]Albumin levels may also be reduced in patients who are malnourished, have cancer, malabsorption, or immune disorders (therefore it lacks specificity).
[d]The increase in time it takes for the blood to clot will depend on the extent of functional damage, and this can vary greatly within conditions.
↑ mild increase; ↑↑ moderate increase; ↑↑↑ marked increase.

patient history can confirm diagnosis (Rosalki *et al*., 1971). However, even in the absence of alcohol, γ-glutamyl transferase can be elevated, as seen in patients with NAFLD, and shows a clinical picture similar to chronic hepatitis (Brunt, 2004). If clinical presentation suggests alcoholic-induced liver disease but without evidence of alcohol consumption, other conditions such as diabetes, hyperlipidaemia, and hypertension should be assessed to assist in a diagnosis of NAFLD. Ultimately, a liver biopsy would be required to ascertain the extent of hepatic injury (Dufour *et al*., 2000a,b).

2.7.2 Moderate/marked elevation of ALT and AST

Acute damage to the liver can also result in a moderate/marked increase in AST and ALT. When levels of AST/ALT reach 100X the URL, it is almost always indicative of ischaemic injury or injury due to toxic ingestion (Dufour *et al*., 2000a,b). Ischaemic and hypoxic acute liver damage are frequently associated with patients who are clinically challenged, such as those with sepsis (Seeto *et al*., 2000). Toxin ingestion can captivate prescribed and herbal medications and intended overdose use. Within several hours of an overdose of acetominophen levels of AST can reach in excess of 7000 IU l^{-1}, reflecting the ultimate destruction of liver cells leading to an immediate release of enzymes into the blood stream (Singer *et al*., 1995). As also seen in ischaemic hepatic injury, the ratio of AST/ALT will be increased and reflect an increase in lactate dehydrogenase, a marker of ischaemic injury. Post insult the levels of AST and ALT can drop dramatically to within the reference range; however, this is not necessarily a good prognosis as it may reflect extensive hepatic necrosis rather than recovery, due to the short circulatory half-life of AST (17 h) and ALT (47 h) (Giannini *et al*., 2005). In this case the monitoring of true liver function tests such as bilirubin levels or prothrombin time can assess if the patient is at risk from hepatic failure.

It is estimated that over 80% of individuals with acute viral hepatitis are never clinically diagnosed (Giannini *et al*., 2005; Knight, 2005). This is mostly due to the asymptomatic presentation of those infected and is often passed off as a flu-like illness with non-specific indicators such as fatigue and fever. Although hepatitis A and hepatitis B are on the decrease worldwide due to the introduction of vaccines, hepatitis C is increasing. For those with hepatitis C, 85% develop chronic hepatitis and have a 30% increased risk of developing hepatocellular cancer. Although jaundice is evident in almost 70% of those with acute hepatitis A, it occurs in less than 50% and 33% of cases with acute hepatitis B and C, respectively, and is therefore an insensitive diagnostic indicator (Dufour *et al*., 2000a,b; Giannini *et al*., 2005). Levels of the aminotransferases can also be varied dependent on the extent of cellular damage, and can show a moderate or marked increase with or without jaundice. As a result differential diagnosis of acute viral hepatitis can be more challenging for viral hepatitis, in particular hepatitis C. Although biochemically the clinical picture may not be clear, patient history and evidence of high-risk factors such as travel to endemic areas (hepatitis A), intravenous drug use, or transfusions will play a pivotal role in diagnosis. Confirmation of acute viral hepatitis, however, is only obtained when the patient is tested for viral-specific antibodies. Although toxin or viral insult represent the vast majority of acute liver diseases with moderate or marked increases in AST and ALT, other causes such as Epstein–Barr virus, autoimmune, extrahepatic, or congenital disorders must be considered (Giannini *et al*., 2005).

2.8 Conclusions and Future Directions

Although the intriguing compartmentation of metabolic pathways and the subcellular localization of the aminotransferase isoforms to different brain cells offer enumerable insights into whole-brain metabolism, they also highlight the enormity of what is not understood. Evidently, the cellular distribution of the aminotransferases in brain tissue requires further characterization to substantiate the biochemical findings. Confirmation of the expression and activity of the specific isoforms in brain

cells will further assist understanding of the actual extent to which these proteins participate in anaplerotic pathways. The current studies unequivocally support the role that these aminotransferase proteins play in the supply of nitrogen for glutamate synthesis. In particular, studies detailing the role of leucine in glutamate oxidation and synthesis show that it fulfils the criteria as an external nitrogen source:

1. It freely passes the blood brain barrier.
2. The BCAT proteins are highly expressed in brain cells showing neuronal and astrocytic specificity.
3. Their role in muscle metabolism as a nitrogen donor is substantially characterized.
4. Overwhelming evidence in several model systems support the BCAA–BCKA shuttle with direct evidence of its contribution to the glutamate–glutamine cycle.

However, as with the other aminotransferase proteins, much is still unknown. More sensitive models using inhibitors with greater specificity would further enhance knowledge of these pathways. As these aminotransferase proteins play a key role in facilitating the anaplerotic generation of glutamate it is highly likely that their metabolism will be altered in neurodegenerative disease conditions. Therefore, understanding of how these suggested shuttles are altered in disease will offer possible targets for novel therapeutic treatment to either delay onset or prevent further neuronal destruction. Finally, to date ALT and AST have for decades sustained their role as key biochemical makers of disease, despite their lack of specificity. With the advent of proteomics and the search for the ideal marker of early stage liver disease, new markers are emerging. However, ALT and AST will sustain their prominent role for the foreseeable future and their value may extend if modifications of these proteins are observed at the early stages of disease.

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