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ORIGINAL PAPER

Branched-Chain Amino Acids and Brain Metabolism

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Abstract This review aims to provide a historical reference of branched-chain amino acid (BCAA) metabolism and provide a link between peripheral and central nervous system (CNS) metabolism of BCAAs. Leucine, isoleucine, and valine (Leu, Ile, and Val) are unlike most other essential amino acids (AA), being transaminated initially in extrahepatic tissues, and requiring interorgan or intertissue shuttling for complete catabolism. Within the periphery, BCAAs are essential AAs and are required for protein synthesis, and are key nitrogen donors in the form of Glu, Gln, and Ala. Leucine is an activator of the mammalian (or mechanistic) target of rapamycin, the master regulator of cell growth and proliferation. The tissue distribution and activity of the catabolic enzymes in the peripheral tissues as well as neurological effects in Maple Syrup Urine Disease (MSUD) show the BCAAs have a role in the CNS. Interestingly, there are significant differences between murine and human CNS enzyme distribution and activities. In the CNS, BCAAs have roles in neurotransmitter synthesis, protein synthesis, food intake regulation, and are implicated in diseases. MSUD is the most prolific disease associated with BCAA metabolism, affecting the branched-chain α-keto acid dehydrogenase complex (BCKDC). Mutations in the branched-chain aminotransferases (BCATs) and the kinase for BCKDC also result in neurological dysfunction. However, there are many questions of BCAA metabolism in the CNS (as well as the periphery) that remain elusive. We discuss areas of BCAA and BCKA metabolism that have yet to be researched adequately.

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Introduction

The BCAAs, Leu, Ile, and Val are nutritionally essential AAs that are necessary for protein synthesis and also important for other metabolic functions (Fig. 1). Branched-chain amino acids are key nitrogen donors involved in interorgan and intercellular nitrogen shuttling, and Leu is an important nutrient signal [1–4]. Unlike most other essential AAs, BCAAs are initially transaminated in extrahepatic tissues via the branched-chain aminotransferases (BCAT) isozymes, followed by irreversible oxidative decarboxylation of the α-keto acid products, catalyzed by the branched-chain α-keto acid dehydrogenase enzyme complex (BCKDC), where liver is thought to be a primary site of oxidation [2, 5].

The CNS requires AA and nitrogen balance, as neurological dysfunction and damage can occur with accumulation or deprivation of AAs, their neurotransmitters, and nitrogen [6]. Accumulation of nitrogen in the form of ammonia in liver failure or imbalance of AA neurotransmitters in the CNS, found in some inborn errors of metabolism, can result in neurologic dysfunction and irreversible brain damage [6]. Sustained deprivation of AA or nitrogen sources can deprive the brain of the necessary AA for protein synthesis and the AA Glu, the major excitatory neurotransmitter, which serves as the precursor to the inhibitory neurotransmitter γ-aminobutyric acid (GABA).

Maintenance of CNS Glu homeostasis is essential, as intra-neuronal Glu concentrations must be sufficient to supply Glu for neuronal depolarization [6]; extracellular Glu must be kept low because sustained elevated

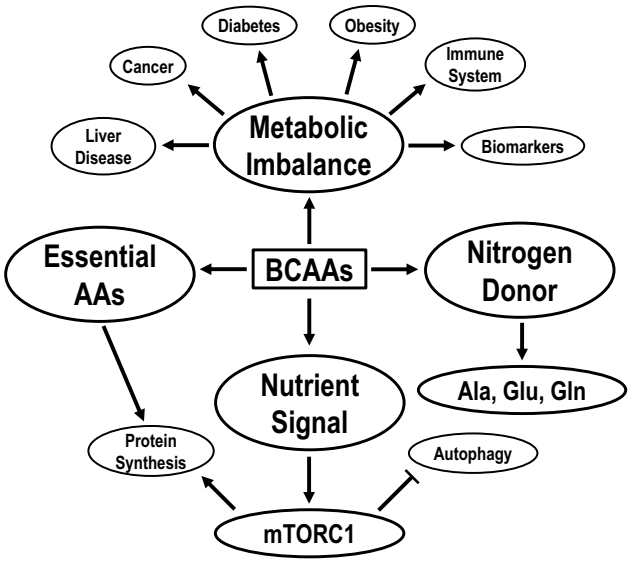

Fig. 1 Roles of BCAAs in peripheral tissues. The BCAAs are nitrogen donors via transamination to produce α-keto acids and Glu, forming Gln via glutamine synthetase (GS). Glutamate nitrogen is transferred to Ala. Alanine and/or Gln are critical for nitrogen disposal from amino acid oxidation in the periphery, gluconeogenesis, and Gln is a major fuel for the intestine. In target tissues such as skeletal muscle, Leu activates mTOR via mTORC1, resulting in increased protein synthesis. Other anabolic pathways such as lipid synthesis and inhibition of autophagy are impacted in a tissue specific manner. BCAAs are essential amino acids, hence they are required for protein synthesis. They are also involved in metabolic homeostasis, stimulate insulin release, may be biomarkers associated with diseases such as diabetes, influence the immune system, and are involved in cancer

extracellular Glu can induce excitotoxic injury [6–8]. To prevent excitotoxic injury, there is limited transport of Glu from the blood to the CNS [6–8]. Studies on AA transport across the blood brain barrier (BBB) have illustrated that the uptake of Glu and Gln (approximately 5%) is less than that of other AAs, whereas the carrier system for neutral AAs at normal blood AA concentrations is more than 50% occupied by Leu and Phe together [9]. Glutamine formed via glial glutamine synthetase (GS) in astrocytes and transported to neurons, is part of the Glu/Gln cycle and is also exported from the brain in exchange for other AAs [6, 10]. Yudkoff postulated that the AAs that supply the amino group for Glu must satisfy the following criteria: (1) the AA must be rapidly transported across the BBB; (2) the donor AA must be non-neuroactive; and (3) the amino group from the donor must be readily transferred to α-ketoglutarate (α-KG) [6]. The BCAAs, particularly Leu, fit these criteria and have been shown to be nitrogen donors in brain for Glu [6].

History of BCAA Peripheral Metabolism

The initial step in the catabolism of BCAAs is reversible transamination of Leu, Ile, and Val with α-KG to produce their respective branched-chain α-keto acids (BCKAs), α-ketoisocaproate, α-keto-β-methylvalerate, and α-ketoisovalerate (KIC, KMV, and KIV, respectively) and Glu via the BCAT isozymes (Fig. 2) [11–13]. The second step is the oxidative decarboxylation of the BCKAs to produce their respective branched-chain acyl-CoA derivatives by the BCKDC (reviewed in [4]). Oxidative decarboxylation of the BCKAs is highly regulated, because it commits the carbon skeleton of these AAs to irreversible catabolism, which permits net transfer of BCAA nitrogen to Glu [4].

In 1966, Ichihara and Koyama determined that all three BCAAs are transaminated by a single enzyme termed BCAT [14, 15]. These researchers went on to describe several other BCATs [16]. In 1976, Shinnick and Harper showed transamination occurs in rodent extrahepatic tissues while BCKDC activity was highest in liver but present in all tissues tested [5]. By 1988, Hutson and colleagues had demonstrated that there are only two BCAT isoenzymes—the mitochondrial $BCAT_m$ and the cytosolic $BCAT_c$ [12, 13]. $BCAT_m$ is the predominant enzyme found in most rat tissues, with liver containing no BCAT activity [13, 17]. $BCAT_c$ is the predominant isozyme that is found in neuronal tissues [18]. Cangiano et al. [19] demonstrated that BCAAs (and other large neutral AAs) readily pass the BBB, and Cooper and Plum [20] proposed that BCAAs are involved in brain nitrogen metabolism and Gln synthesis [19, 20].

The BCKDC is structurally similar to both the pyruvate dehydrogenase enzyme complex and the α-KG dehydrogenase enzyme complex, with which it shares functional homology [21]. The BCKDC consists of multiple copies of three enzymes: (1) branched-chain α-keto acid dehydrogenase (E1—12 copies); (2) dihydrolipoyl transacylase (E2—24 copies); and (3) dihydrolipoyl dehydrogenase (E3–6 copies). The latter is common to all three dehydrogenase complexes [21]. BCKDC activity is controlled via its phosphorylation state of the E1 subunits, with the phosphorylated form rendering the complex inactive [22, 23]. Flux of BCKAs through BCKDC generates branched chain acyl-CoAs, which undergo further catabolism for entry into the TCA cycle, or serve as precursors for lipogenesis and gluconeogenesis. Maple syrup urine disease (MSUD) results from one or more functional mutations in the BCKDC E1 and E2 protein components (reviewed in [24]).

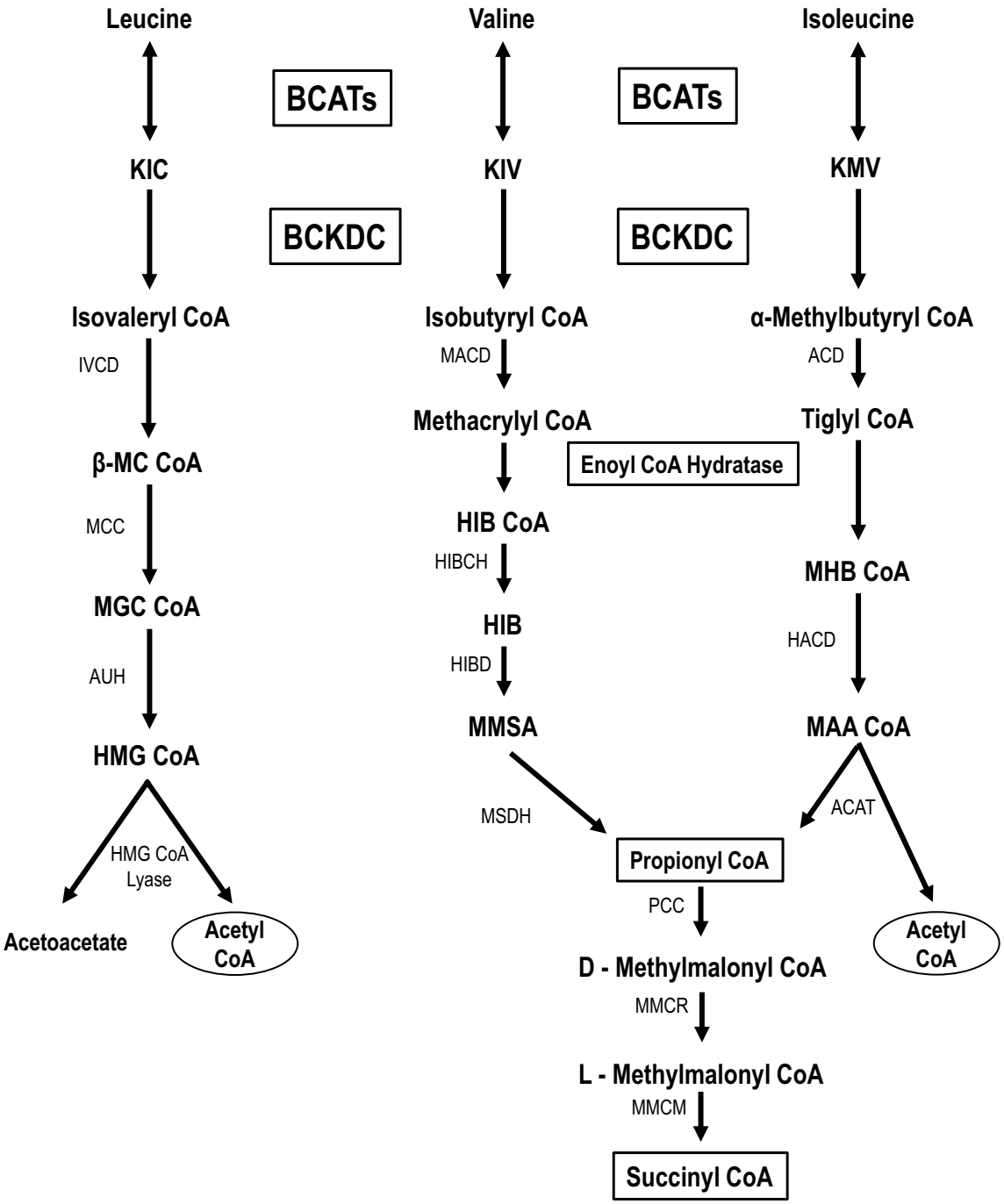

Fig. 2 BCAA catabolic pathway. Transamination by branched-chain aminotransferase isozymes (BCATs) and oxidative decarboxylation by branched-chain α-keto acid dehydrogenase complex (BCKDC) are the initial common steps in BCAA catabolism. Valine and Ile also share Enoyl CoA Hydratase. Leucine is solely ketogenic, producing acetoacetate and acetyl CoA. Valine is solely gluconeogenic, producing Succinyl CoA. Isoleucine is both ketogenic and gluconeogenic, producing acetyl CoA and Succinyl CoA. *KIC* α-ketoisocaproate, *IVCD* isovaleryl CoA Dehydrogenase, *β-MC CoA* β-methylcrotonyl CoA, *MCC* β-methylcrotonyl CoA carboxylase, *MGC CoA* β-methylglutaconyl CoA, *AUH* β-methylglutaconyl CoA hydratase, *HMG CoA* β-hydroxy-β-methylglutaryl CoA, *HMG CoA Lyase* β-hydroxy-β-methylglutaryl CoA lyase, *MACD* α-methyl acyl CoA dehydrogenase, *HIB CoA* β-hydroxyisobutyryl CoA, *HIBCH* β-hydroxyisobutyryl CoA hydrolase, *HIB* β-hydroxyisobutyrate, *HIBD* β-hydroxyisobutyrate dehydrogenase, *MMSA* methylmalonate semialdehyde, *MSDH* methylmalonate semialdehyde dehydrogenase. *PCC* propionyl-CoA carboxylase, MMCR methylmalonyl CoA racemase, *MMCM* methylmalonyl CoA mutase, *KMV* α-keto-β-methylvalerate, *ACD* acyl CoA dehydrogenase, *MHB CoA* α-methyl-β-hydroxyisobutyryl CoA, *HACD* β-hydroxyacyl CoA dehydrogenase, *MAA CoA* α-methylacetoacetyl CoA, *ACAT* acetyl CoA acyl transferase

Peripheral BCAA Catabolism Involves Interorgan Shuttling of BCAA Metabolites

The enzymes responsible for the degradation of BCAAs are found in tissues throughout the body and usually require the shuttling of metabolites for complete catabolism (Fig. 3) [5, 25, 26]. Adult rodent liver contains no BCAT, therefore the BCAAs pass through the liver unaltered; in skeletal muscle, BCAAs account for approximately 50% of the AA uptake [27–30]. In the rat, $BCAT_m$ is expressed in most tissues while $BCAT_c$ is limited to the brain, peripheral nervous system, ovaries, and placenta [17, 18]. In the liver, BCKDC activity is high, with moderate activity in the kidney and heart, and limited active enzyme in skeletal muscle [31–33]. In human tissues, expression of BCAT and BCKDC was, with a few exceptions, comparable to rat tissues, with some $BCAT_m$ observed in Kupffer cells in primate liver [2, 34]. The interorgan/tissue shuttling of BCKA (and other metabolites) for complete oxidation allows for nitrogen transfer and oxidation to occur in different tissues or cells within a tissue, depending on their need for energy, metabolites, or AAs (Fig. 3).

The α-amino group of Glu itself is often transferred to another α-keto acid via separate aminotransferases. The most important metabolically are Asp aminotransferase (AST) and Ala aminotransferase (ALT) with recipient α-keto acids oxaloacetate and pyruvate, respectively [4]. Amidation of Glu by GS produces Gln [25]. About 60% of the nitrogen released by muscle is in the form of Ala and Gln [25, 28–30]. Alanine transports nitrogen to the liver for disposal via the urea cycle, and carbon (as pyruvate) also is transported to the liver for use in gluconeogenesis [35]. Glutamine serves to provide ammonia to the kidney for maintenance of acid-base balance, is a major energy source for the intestine and other tissues (for example some lymphocytes), and is important for body ammonia balance in liver and other tissues [36]. Glutamine is also a precursor to key compounds including nucleic acids and amino sugars.

Because Glu and Asp are approximately 17% of muscle AA uptake and Ala is released rather than taken up from muscle, amino acid metabolism, particularly

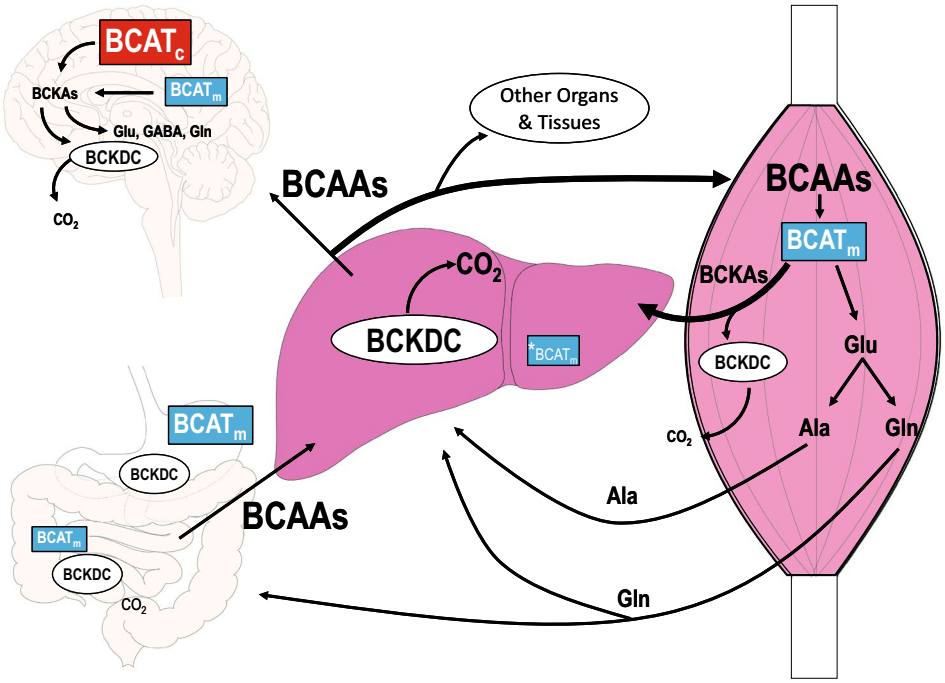

Fig. 3 Liver and skeletal muscle play a major role in interorgan shuttling of BCAA nitrogen whereas in brain, intercellular shuttling predominates. Ingested BCAAs that are not used by the liver are delivered to extrahepatic tissues. In the liver, there is high BCKDC activity without BCAT activity. The exception (*$BCAT_m$) is human and non-human primate liver where $BCAT_m$ is found at low levels, but not in hepatocytes with BCKDC. In skeletal muscle, BCAAs are transaminated by $BCAT_m$ to produce α-keto acids (BCKAs) and Glu. A significant fraction of the BCKAs are released into the circulation. Liver is a primary site of oxidation, though oxidation and transamination can occur in many tissues. Glutamine is transported to the liver where it is involved in nitrogen homeostasis, is fuel for the intestine, and other cells. Alanine is transported to the liver for gluconeogenesis and the nitrogen is disposed in the form of urea. The brain contains both BCAT isoenzymes and BCKDC. In humans, $BCAT_m$ and BCKDC are located in the endothelium of the BBB and $BCAT_c$ is located in the neuronal populations. In the rodent brain, $BCAT_m$ is located in the astrocytes and $BCAT_c$ exhibited a neuronal location. *$BCAT_c$* cytosolic branched-chain aminotransferase, *$BCAT_m$* mitochondrial branched-chain aminotransferase, *BCKDC* branched-chain α-keto acid dehydrogenase enzyme complex, *BCKAs* branched chain α-keto acids

BCAA transamination, supplies nitrogen for muscle Ala, Glu, and Gln pools [4]. Skeletal muscle BCKDC is less active than BCKDC in tissues such as liver, kidney, and heart [4]. Recently, a study restricting dietary BCAAs in Zucker-lean and -fatty rats [37] showed that inhibition of hepatic BCAA oxidation in the obese animals drives more BCAAs, Phe, and Tyr into skeletal muscle. Skeletal muscle BCAA oxidation was elevated resulting in the accumulation of fatty acyl-CoAs, with consequent incomplete lipid oxidation, depletion of Gly/Ser, and skeletal muscle insulin resistance [37]. The results of this study and others indicate the importance of the coordinated regulation of BCKDC by BDK and the phosphatase (Protein Phosphatase, Mg^{2+}/Mn^{2+}-dependent 1K; PPM1K) in different organs and tissues and the impact of the nutritional and hormonal milieu in coordinate regulation of the catabolic enzymes for normal homeostasis as well as the consequences of hormonal dysregulation. As the above example with the Zucker fatty rat indicates, alterations of the BCAA catabolic pathway, hence BCAAs and their metabolites, is associated with obesity, diabetes, cancer, and recently immune system function [37–41]. Thus, it is not surprising that BCAAs have an important role in CNS metabolism as well.

CNS BCAA Metabolism also Involves Metabolite Shuttling

The function of BCAA in peripheral nitrogen metabolism, the significant brain uptake of BCAA, and the neurologic effects on the glutmatergic and GABAergic system observed in a spontaneously occurring bovine MSUD model, support the hypothesis that they are nitrogen donors in Glu and Gln metabolism in the brain [6, 42, 43]. Glutamate is the major excitatory neurotransmitter in brain, and it serves as a precursor for Gln and the major inhibitory neurotransmitter GABA [6, 42]. The Glu/Gln cycle, first described by Berl and Clark (in ref. [44]) using rodent models involves astrocytes and neurons in the brain, regulates neuronal Glu stores, and prevents excess Glu in the synaptic cleft (rodent – Fig. 4; human – Fig. 5) [6, 42, 45]. Not all Glu is removed by the postsynaptic neuron during neurotransmission, and astrocytes surrounding neurons express a large concentration of Glu-specific transporters to remove excess Glu. Astrocytic GS converts Glu to the non-neuroactive Gln. Glutamine is then released to the extracellular fluid for reuptake by the pre-synaptic neuron. Within the pre-synaptic neuron, Gln is converted back to Glu via glutaminase (GLA), completing the Glu/Gln cycle [6, 42, 45].

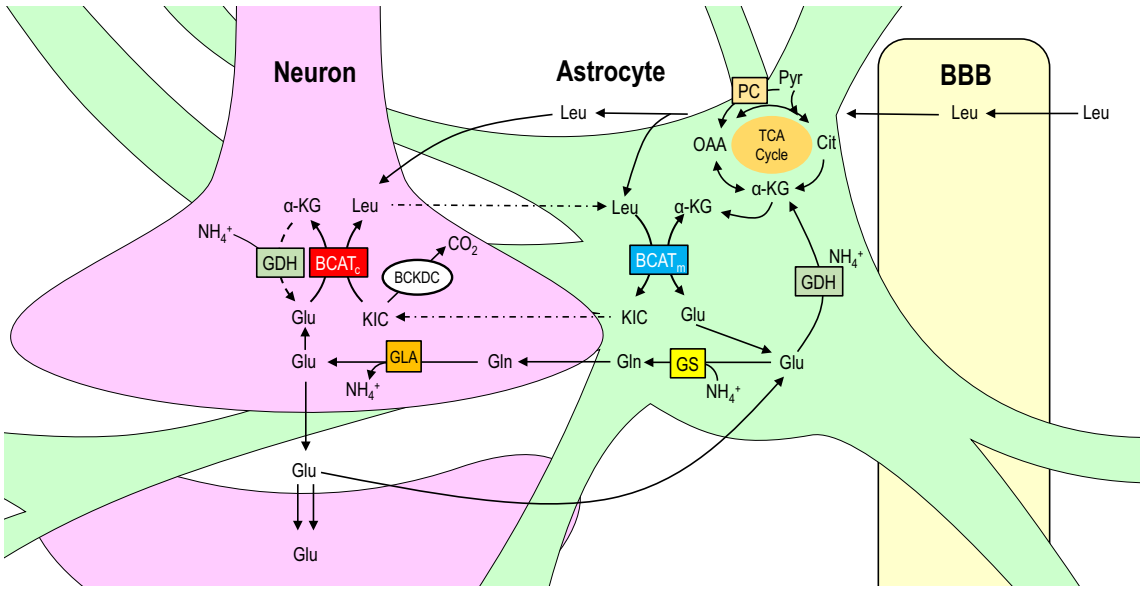

Fig. 4 Rodent brain nitrogen transfer. Leucine is readily taken up by the BBB for delivery to astrocytes (and neurons). In astrocytes, Leu is transaminated by $BCAT_m$ to produce Glu and KIC. α-KG comes from de novo synthesis to replace Glu carbon lost by oxidation in the TCA cycle. KIC is transported to the neuron and Glu is converted to Gln for transport to the neuron (Glu/Gln cycle). In the neuron, Gln is converted to Glu for neurotransmission. $BCAT_c$ completes the Leu cycle by transfer of nitrogen from Glu to KIC to form Leu and α-KG. Leucine returns to the astrocyte. The α-KG is then aminated via GDH to reform Glu. The cycle transfers nitrogen to neuronal Glu and the $BCAT_c$ arm alone can buffer Glu in the neuron (prevent excitotoxic build up). *$BCAT_c$* cytosolic branched-chain aminotransferase, *$BCAT_m$* mitochondrial branched-chain aminotransferase, *BCKDC* branched-chain α-keto acid dehydrogenase enzyme complex, *GLA* glutaminase, *GS* glutamine synthase, *KIC* α-ketoisocaproate, *α-KG* α-ketoglutarate, *GDH* glutamate dehydrogenase, *PC* pyruvate carboxylase

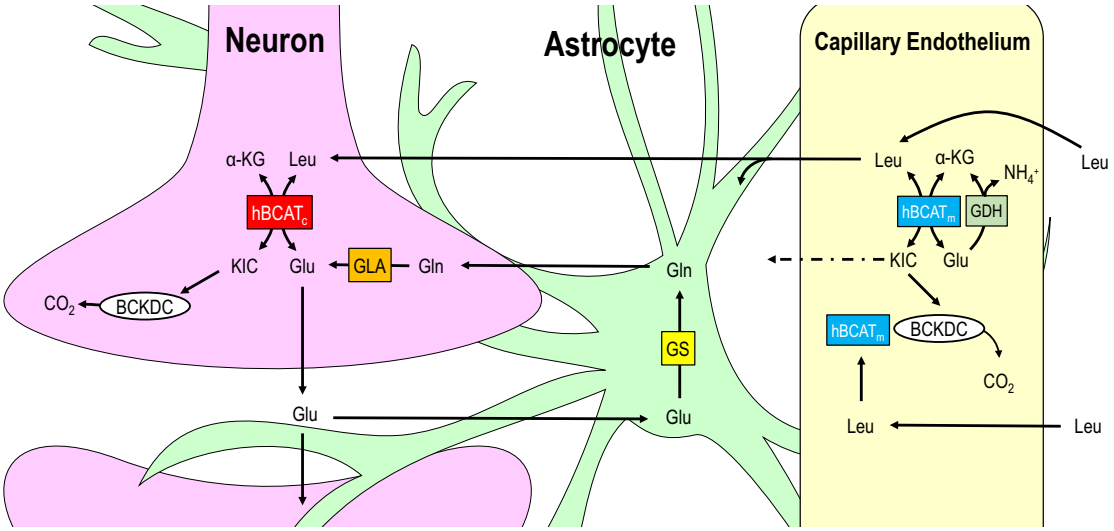

Fig. 5 Human brain nitrogen transfer. Leucine is readily taken up by the BBB for transamination or delivery to neurons. Oxidation and transamination of BCAAs can occur within the endothelium of the BBB catalyzed by the mitochondrial $BCAT_m$ and BCKDC or directly by the $BCAT_m$/BCKDC metabolon. Alternatively, a $BCAT_m$/GDH metabolon could release ammonia without loss of mitochondrial α-KG. In the neuron, Leu is transaminated by $BCAT_c$ to produce Glu and KIC. Glutamate, the major excitatory neurotransmitter, is released into the synaptic cleft for neurotransmission. Glutamate not taken up by the post-synaptic neuron is sequestered in the astrocyte for conversion to non-neuroactive Gln and transported to the neuron. In the neuron, Gln is then converted to Glu. *$BCAT_c$* cytosolic branched-chain aminotransferase, *$BCAT_m$* mitochondrial branched-chain aminotransferase, *BCKDC* branched-chain α-keto acid dehydrogenase enzyme complex, *GLA* glutaminase, *GS* glutamine synthase, *KIC* α-ketoisocaproate, *α-KG* α-ketoglutarate, *GDH* glutamate dehydrogenase

The Glu/Gln cycle accounts for approximately 80% of Gln synthesis but a portion of the neurotransmitter Glu is lost to oxidative metabolism in the astrocyte [46–48]. Astrocytes express the anaplerotic enzyme pyruvate carboxylase (PC; Fig. 4), which is essential for replenishing the Glu carbon skeleton (α-KG) that is lost via oxidative metabolism [42, 46]. The BCATs are highly active in brain, with both $BCAT_m$ and $BCAT_c$ present; evidence that BCAA are involved in Glu synthesis came from studies using primary astrocyte cultures and isolated rat retinas [49–51].

Yudkoff and colleagues used [^{15}N]Leu and [^{15}N]Val in organotypic cerebellar explants of newborn mouse cerebella to determine the fraction of α-nitrogen incorporation into Glu or Gln, and reported that approximately 25% of Glu nitrogen was formed from Leu, approximately 4% derived from Val, and incorporation of all three BCAAs was estimated to account for about 1/3 of Glu nitrogen [52]. Primary astrocyte cultures incubated in media containing [^{15}N]Leu and an additional 15 non-labeled AAs showed that after 3 hours of incubation, the ratio of [^{15}N]Glu/[^{15}N]Leu was 0.21, indicating that at least 1/5 of all Glu nitrogen is derived from Leu [53]. A BCAA cycle with Leu transamination in astrocytes followed by KIC release and reamination in neurons with subsequent Leu return to astrocytes was proposed as an adjunct to the Gln/Glu cycle (see in Fig. 4) [49, 54, 55]. In this cycle, neuronal Glu was derived from the action of GLA and the reductive amination of α-KG by glutamate dehydrogenase (GDH). One caveat to this cycle is that the energetically preferred reaction for GDH is oxidative deamination of Glu to form α-KG, and unless Glu is very high, transamination of Leu to form Glu and KIC is preferred [56]. On other hand, neuronal Glu concentrations are higher than in other cells and GDH activity is influenced by redox state, therefore cycling is possible.

Not all of the Glu taken up by the astrocyte is converted directly to Gln; some of the Glu is oxidized in the TCA cycle resulting in loss of the Glu carbon skeleton. LaNoue and colleagues [46, 49, 50] were able to show that the lost carbon skeleton is replaced via de novo Glu synthesis primarily catalyzed by PC [46, 49, 50]. Hutson and colleagues [49–51, 57–59] developed a version of the BCAA cycle in rodent brain that integrates the cellular localization of the BCAT isozymes to provide nitrogen for Glu and Gln synthesis and the role of astrocytes in de novo Glu synthesis (Fig. 4) [49–51, 57–59]. In this BCAT cycle, Leu transamination in the astrocyte is catalyzed by $BCAT_m$ and neuronal transamination is catalyzed by $BCAT_c$ [49]. Evidence for a BCAT specific cycle in rodents came from immunohistochemical localization of brain $BCAT_c$ in glutamatergic and GABAergic neurons and $BCAT_m$ in astroglia. Gabapentin, a neuroactive drug with structural resemblance to Leu, was found to be a competitive inhibitor of $BCAT_c$ and inhibited de novo Glu synthesis in isolated rat retina [49].

In vivo studies using ^{15}N provided evidence that at physiological concentrations of blood and brain Leu, approximately 20–25% of nitrogen transfer in rodent brain was derived from Leu [60–62]. Using ^{13}C-NMR, de novo synthesis of Glu was calculated to be approximately 20% of Gln/Glu cycle flux, which agreed with the measures performed in isolated rat retina [51]. Subsequently, Rothman et al. [63] analyzed several proposed AA shuttles using kinetic models developed using NMR to measure TCA cycle flux and Glu/Gln cycle flux. They concluded that the BCAT cycle could contribute to de novo Glu synthesis, but noted that there was limited evidence for GDH catalyzing Glu synthesis (forward reaction), which is required for the shuttle to operate in the direction shown in Fig. 4 [63]. Certainly, if neuronal Glu is high enough and the redox state favorable, it could drive $BCAT_c$ toward Leu formation and buffer Glu in the neuron, where Glu can exceed 10 mM. Both BCATs have K_m values for Glu that are similar to neuronal Glu concentrations, and $BCAT_c$ is located in the neuronal processes [58]. In conclusion, there is a consensus that BCAA contribute nitrogen to Glu synthesis in the rodent brain, however, the existence of a strict BCAT shuttle remains to be proven unequivocally [64, 65].

There are striking differences between the rodent and human brain with respect to the location of $BCAT_m$; it is located in the capillary endothelial cells and not in astrocytes (Fig. 5) [64, 65]. Interestingly, Conway and colleagues (unpublished data) have shown that BCKDC and GDH are also present in the endothelium, presenting the possibility that the GDH/$BCAT_m$ and $BCAT_m$/BCKDC metabolons, as described by Hutson and colleagues [66–68], may form in the mitochondria [64, 66–68]. Metabolons allow for direct channeling of substrates and products between enzymes in the complex without release into the cellular fluid. $BCAT_m$, but not $BCAT_c$, binds and forms a metabolon with GDH or BCKDC. The $BCAT_m$/GDH metabolon facilitates oxidative deamination of Glu formed during $BCAT_m$ catalyzed transamination of BCAA by GDH, regenerating α-KG and releasing NH_4^+, eliminating loss of the TCA cycle intermediate α-KG, and generating the NH_4^+ for Gln synthesis if diffusion into the astrocyte can occur or if Gln is released into the blood [64]. If the $BCAT_m$/BCKDC metabolon formed, the KIC product of $BCAT_m$ would be channeled to BCKDC for oxidation with net transfer of Leu nitrogen to Glu. Formation of both metabolons is influenced by redox state of $BCAT_m$ [66–68]. Oxidation of the reactive CXXC center in $BCAT_m$ results in inhibition of $BCAT_m$ activity and inhibits metabolon formation [64]. Thus, in the human brain, the capillary endothelium may take on some of the functions of the astrocyte in rodent brain. The ability of the capillary endothelial cells to contribute to BCAA N-transfer will depend on mitochondrial transport of BCAAs and BCKAs, redox state, and the actual concentrations of these metabolites in the cells.

Leucine is an Anabolic Nutrient Signal Activating mTOR

Another role for the BCAA Leu, which is relevant to brain, is activation of mTOR, a serine/threonine protein kinase involved in the control of cell growth and proliferation [69]. mTOR exists in two distinct protein complexes: mTORC1 and mTORC2 [69–71]. mTORC1 is rapamycin-sensitive and mTORC2 is rapamycin-insensitive [69–71]. mTOR is involved in multiple brain functions, including synaptic plasticity, metabolic regulation, axon and dendrite development, neuron differentiation, gliogenesis, and food intake regulation [69, 72]. The BCAA Leu has long been known as a signaling molecule that stimulates protein synthesis in the periphery by activation of the mTORC1 pathway in selected tissues, particularly skeletal muscle [1, 73, 74].

mTORC1 is sensitive to AAs and regulates growth by controlling protein and lipid synthesis, as well as autophagy [75–77]. mTORC1 requires a bipartite mechanism of activation through the Rag and Rheb GTPases at the lysosome [76, 77]. Growth factors and energy levels exert control of the subcellular localization of mTORC1 through Rheb GTPases, while nutrient inputs exert control via the Rag GTPases [76, 77]. Both inputs are needed for the complete activation of mTORC1, as the absence of one inhibits mTORC1activation [76, 77].

The tRNA charging enzyme, leucyl tRNA-synthetase (LRS), is thought to be the Leu signal for mTORC1 activation [78, 79]. Indeed, in mammals, the accumulation of Leu in the cytosol induces the translocation of the LRS to the lysosome, where it interacts with Rag D and facilitates GTP hydrolysis [78, 79]. Rag B/Rag D form a heterodimer, and the GTP hydrolysis of Rag D and GTP loading of Rag B are necessary for the activation of mTORC1 [78, 79]. GTP loading of the Rag A or Rag B recruit mTORC1 to the Ragulator-Rag GTPase docking site at the lysosomal surface, where it interacts with the small GTPase Rheb for mTORC1 activation [77].

Leu Affects Food Intake via mTORC1

Administration of a bolus of Leu into the third ventricle induced mTORC1 activity in the hypothalamus, resulting in decreased food intake and body weight in rats [75]. The hypothalamus is the signaling center linking peripheral organ metabolism to CNS action, relaying circulating hormone and nutrient signals to neuroendocrine cells for homeostatic regulation [80]. mTORC1 is highly

expressed in the CNS, but is restricted in the hypothalamus to the arcurate nucleus, ventromedial hypothalamus, paraventricular hypothalamus, and the suprachiasmatic nucleus [80, 81]. The arcurate nucleus contains two subpopulations of neurons involved in satiety—either orexigenic (neuropeptide Y and agouti-related peptide; NPY and AgRP) or anorexigenic (proopiomelanocortin and cocaine-and-amphetamine-regulated transcript; POMC and CART) [75, 80, 81].

Leucine stimulation of mTORC1 resulted in decreased NPY and AgRP expression and increased POMC expression within the arcurate nucleus [75, 80]. Similarly, the appetite-suppressing hormone leptin also acts on POMC and AgRP neurons [75]. Overnutrition resulted in decreased mTORC1 activity in the hypothalamus, and was linked to hyperphagia and weight gain, along with high-fat diet induced obesity and leptin resistance, both conditions where BCAA catabolism is impaired and circulating BCAAs are elevated [82]. AMP-activated protein kinase (AMPK) is a known modulator of cellular metabolism in response to energy demand via allosterically rising AMP levels and is an inhibitor of mTORC1 activity [83]. Leucine-induced food intake suppression was dependent on mTORC1 activity and inhibition of AMPK [83].

Leucine may also influence peripheral hormonal signals. The rise of circulating leptin following a meal was partly dependent on the presence of Leu in the food [82, 84]. Indeed, a Leu-deficient meal decreased leptin secretion by approximately 40% in rats [82, 84]. Leucine modulation of leptin secretion required increased mTORC1 activity in adipose tissue to increase plasma leptin, and administration of rapamycin inhibited the leptin peak [82, 84].

However, not all are in agreement that Leu affects hypothalamic regulation of food intake. In oral chronic Leu supplementation in rats, Zampieri et al. [85] did not observe any significant reduction in food intake or changes in hypothalamic gene expression [85]. Leucine supplementation did increase markers of mTORC1 activation within the hypothalamus, but only activated neurons of the area postrema of the brainstem, an area of chemoreception in the detection of toxins and controlling nausea and vomiting [85]. Lesions of the area postrema also prevent taste-aversion, and with Leu supplementation via oral gavage activating neurons in the area postrema, it is possible that the area postrema is involved in the CNS ability to sense Leu [85]. Nevertheless, both the taste-aversion to Leu and whether Leu is effective at physiologically relevant concentrations need further investigation.

Neurologic Consequences Occur When BCAA Catabolism is Blocked or Accelerated

There are metabolic consequences when BCAAs are in excess or are limiting. All three BCAAs are essential AAs, and dietary limitation can affect protein synthesis and other metabolic processes. Conversely, excess BCAAs and their metabolites are associated with neurological dysfunction and acidemias. The most studied inborn error of BCAA metabolism is MSUD (OMIM 248600) [64]. It is an autosomal recessive inborn error of metabolism caused by functional mutations in mitochondrial BCKDC, resulting in the accumulation of BCAAs and their corresponding BCKAs in tissues and plasma [64, 86]. MSUD can be lethal, presenting as episodes of intoxication and acute neurological deterioration [64, 86]. Functional mutations of other enzymes in the catabolic pathway (Fig. 2) have been described, with the presence of neurological dysfunction being a common theme [87].

MSUD results from mutations in the E1α or E1β subunit proteins of E1 enzyme or E2 enzyme protein of BCKDC [24]. During metabolic decompensation, which usually occurs during illness or a febrile episode, increased protein degradation occurs and the pathological rise in BCAA and BCKA results in symptoms which include encephalopathy, cerebral edema, and death [88]. Newborn screening allows for early diagnosis and dietary treatment. Most patients do well with dietary management [24, 64], though there still appear to be neurological effects even in patients with good dietary control [88]. Liver transplantation has been employed recently in the treatment of MSUD. Following transplant, patients appear to be able to tolerate a normal diet and have improved levels of plasma BCAAs [24]. Published data indicate that providing 9–13% of the bodies' BCKDC activity is adequate in the restoration of BCAA homeostasis [24, 64, 89]. Furthermore, success of MSUD liver transplants into non-MSUD patients illustrates how interorgan shuttling of BCAA metabolites facilitate oxidation in extrahepatic tissues in non-MSUD patients receiving a MSUD liver [24].

Progress has been made in elucidating the biochemical mechanisms underlying the toxicity of Leu and KIC (Val, Ile, and their corresponding α-keto acids have little apparent toxicity) [64, 86, 90]. The $BCAT_m$-null mouse has levels of plasma and tissue BCAAs observed during metabolic decompensation in MSUD without the consequent elevation of BCKAs, and did not present with an MSUD phenotype (in ref. [64]). On the other hand, a case of hypervalinemia and hyperleucine-isoleucinemia due to mutations in $BCAT_m$ (BCAT2 gene) has been reported [91]. This patient did experience mild memory impairment and headaches until treated with vitamin B6 (PLP; pyridoxal phosphate), suggesting the chronic elevation of BCAAs does affect

brain function, but the biochemical basis is unknown [91]. Insight into the molecular basis of this inborn error come from the crystal structures of BCATs. As seen from the crystal structures of BCATs in the protein database, both Glu237Lys and Arg143Gln mutations are in the active site, affecting the PLP/PMP (PMP; pyridoxamine phosphate) cofactor binding site, as well as alterations in the size of the BCAA binding pocket (Fig. 6) [92–95].

According to Yennawar et al. (unpublished data), the crystal structures show Glu237 interacts with the pyridine ring nitrogen atom on the PLP cofactor, bestowing a needed positive charge on it as the enzyme proceeds from enzyme-PLP Schiff base to the PMP form during transamination. The Glu237Lys change in charge (negative to positive) and the change in size caused by a longer Lys compared to Glu could weaken PLP/PMP cofactor binding and reactivity. Thus, supplementation of Vitamin B6 shifts the equilibrium towards more co-factor binding leading to better transamination catalysis. The major determinant of the enzyme's substrate and stereo-specificity for L-BCAAs is a group of hydrophobic residues that lock the side chains in place by a number of van der Waals interactions. Arg143 (Fig. 6) is one of the crucial sidechains at the bottom of the substrate binding pocket. Arg143 helps define the optimal size through its hydrogen bond interactions with the side chains of Tyr141 and Tyr70. In the Arg143Gln mutation, the smaller Gln side chain compared to Arg may break these interactions resulting in a larger substrate pocket. Of all the three BCAAs, the smaller Val would be affected most and could exhibit weaker binding leading to hypervalinemia in patients. Arg143 (with Tyr141 and Tyr70) also helps with suitable substrate orientation in the second half of the transamination pathway. The hydrogen bonding is seen in a model of α-KG (PDB codes 5E25, 3UYY). In this model, the α-KG side chain carboxylate has strong salt bridge interactions with Arg143. Hence Arg143Gln mutation could hamper by perturbing the size as well as the electrostatic interaction surface of the substrate binding pocket.

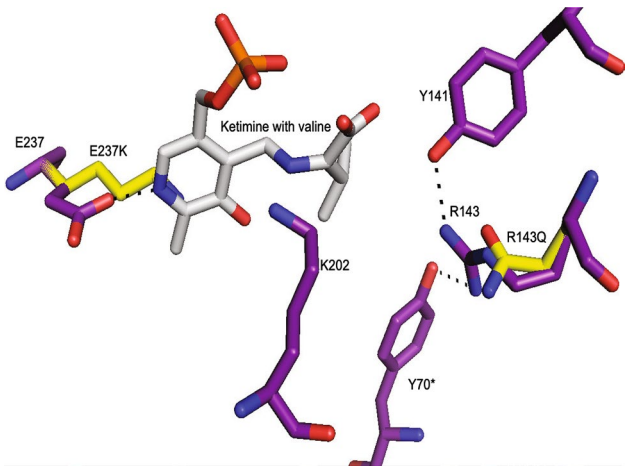

Fig. 6 BCAT mutations. Active site Lys is marked K202. E237K and R143Q mutations shown in yellow are overlaid on the crystal structure of human mitochondrial branched chain aminotransferase (PDB code 1kt8) in purple. Y70* is from the second subunit. A model of the Val ketamine intermediate is depicted in grey. Hydrogen bonds are depicted with *dotted black lines*. *E* glutamic acid, *K* lysine, *Y* tyrosine, *R* arginine, *Q* glutamine

Oxidative stress has been implicated as a mechanism for neurological damage in MSUD patients. Glial cell (astrocytes are a type of glial cell) accumulation of BCAAs is associated with morphological and cytoskeletal alterations [96]. C6 glioma cells are normally round, and exposure to BCKA result in fusiform or process-bearing cells, whereas similar concentrations of BCAAs showed cytoskeletal rearrangement and cell death, with Val having the most profound effects [97]. These changes were accompanied by reduced glutathione (GSH) and significantly increased nitric oxide production [97]. Because both BCATs are inhibited by oxidation of their reactive Cys residues, oxidative stress would likely inhibit transamination and result in a further rise in BCAAs [98, 99]. Administration of antioxidant GSH and N^{ω}-nitro-L-arginine methyl ester (L-NAME) prevented the morphological and cytoskeletal alterations [97]. BCKA treatment resulted in decreased non-enzymatic antioxidant defenses, with KIC also reducing the activity of superoxide dismutase and glutathione peroxidase; BCKAs also elicited nitric oxide production and protection was afforded by addition of antioxidants [100]. It seems likely that structural alterations and oxidative damage do contribute to the functional effects of MSUD on brain development and function; however, these studies used supra-physiological concentrations of a single BCAA or BCKA which does not mimic the in vivo situation.

While excess BCAAs and BCKAs have clear clinical consequences, the converse is also true. Clinical cases showed functional mutations in the branched chain α-keto acid dehydrogenase kinase (BDK; unrestricted BCAA oxidation), resulted in an autism-spectrum disorder with epilepsy [101, 102]. The patients presented with intellectual disability, psychomotor delay, severe developmental delay, behavioral abnormalities, and either seizures or abnormal electroencephalograms (EEG) [101, 102]. BDK disorders can be treated with high-protein diets or BCAA supplementation [102]. As in the BDK knockout mouse, which also has seizures, low or the absence of BDK activity resulted in uninhibited oxidation of BCAAs, creating an essential amino acid deficiency, which impaired protein synthesis during development and postnatal growth [103–105].

Future Directions

It is accepted that BCAAs are essential in interorgan and tissue nitrogen shuttling, and can provide nitrogen for CNS neurotransmitter synthesis. Evidence from studies of inborn errors of metabolism and disease states also suggests that the shuttling of BCAA metabolites is essential for normal physiological function. However, questions still remain, particularly with BCKA intercellular and interorgan shuttling with respect to normal homeostasis and disease, i.e., can metabolite shuttling contribute to disease, ameliorate it, or do both? The data presented in White et al. [37] suggest shuttling can be both beneficial and exacerbate the disease process, as well as affect metabolism of other amino acids, such as Gly and Ser, that are important for methylation reactions.

Although BCAA do contribute nitrogen to Glu (Gln, GABA) in brain, the existence of an obligatory shuttle needs to be verified, i.e., to what extent do $BCAT_m$ in astrocytes and $BCAT_c$ act in concert (i.e., shuttle) or act independently in rodents? In humans, what is the role of the capillary epithelium? The presence of $BCAT_m$ and BCKDC as well as GDH in these cells suggests the potential for oxidation, but whether or not there is a quantitative role for the endothelium needs to be determined. Does the co-localization of these enzymes mean that their metabolons operate in vivo? Furthermore, significant metabolism of BCAAs in the capillary epithelium has the potential to limit BCAA uptake by the brain by reducing substrate supply. What is the biochemical basis and consequences of changes in expression of the BCATs in disease states [65, 106]? What are the critical stages of development where excess/deficiency of BCAAs interfere with normal protein synthesis in the CNS, and do the acyl-CoAs and acetyl-CoAs from BCAA catabolism contribute to myelin synthesis? Finally, little is known about the role of these amino acids and enzymes in the spinal cord and peripheral nervous system, particularly $BCAT_c$, which is localized in peripheral nerves [57].

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