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Interactions in the Metabolism of Glutamate and the Branched-Chain Amino Acids and Ketoacids in the CNS

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Abstract Glutamatergic neurotransmission entails a tonic loss of glutamate from nerve endings into the synapse. Replacement of neuronal glutamate is essential in order to avoid depletion of the internal pool. In brain this occurs primarily via the glutamate-glutamine cycle, which invokes astrocytic synthesis of glutamine and hydrolysis of this amino acid via neuronal phosphate-dependent glutaminase. This cycle maintains constancy of internal pools, but it does not provide a mechanism for inevitable losses of glutamate N from brain. Import of glutamine or glutamate from blood does not occur to any appreciable extent. However, the branched-chain amino acids (BCAA) cross the blood–brain barrier swiftly. The brain possesses abundant branched-chain amino acid transaminase activity which replenishes brain glutamate and also generates branched-chain ketoacids. It seems probable that the branched-chain amino acids and ketoacids participate in a "glutamate-BCAA cycle" which involves shuttling of branched-chain amino acids and ketoacids between astrocytes and neurons. This mechanism not only supports the synthesis of glutamate, it also may constitute a mechanism by which high (and potentially toxic) concentrations of glutamate can be avoided by the re-amination of branched-chain ketoacids.

Keywords Branched-chain amino acids · Brain · Metabolism · Glutamate · Brain metabolism

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Brain Handling of Glutamate: the Glutamate-Glutamine Cycle

The overriding goal of intermediary metabolism is to assure constancy of energy supply and metabolite concentrations. To this end, a dense skein of membrane transporters, enzyme systems and regulatory factors work in a coordinated manner to maintain thermodynamic equilibrium and to avoid potentially toxic elevations of metabolite levels.

Maintaining a constant biochemical milieu poses special challenges for the brain, a metabolically intense system which is characterized by continual alterations of membrane potential and ionic gradients, the abrupt release (and reuptake) of neurotransmitters and dramatic fluctuations in regional blood flow. This dynamic system must articulate with the external circulation in a tightly controlled manner. Thus, the blood–brain barrier admits a limited repertoire of compounds in order to minimize toxic exposures and to assure that endothelial cells expend energy solely for import of essential nutrients. Conversely, brain conserves those essential resources—neurotransmitters such as glutamate are an example—which the system needs to transfer information between neurons. Brain concentrates these compounds within a series of intracellular compartments in order to maximize the signal-to-noise ratio upon their release into the synapse and to avoid the neurotoxicity which accompanies an untoward rise in the extracellular concentration.

Our current understanding of glutamate as a neurotransmitter recapitulates each of these themes. We have long known not only that glutamate depolarizes neurons [25, 44], but that it is, indeed, the primary excitatory neurotransmitter [24, 67]. Furthermore, since the seminal studies of Berl et al. [6] it has been evident that brain glutamate handling is not enacted in a single, metabolically homogeneous pool,

but in a multitude of discrete anatomic and metabolic pools that exchange pivotal metabolites. Insight into the anatomy of this multi-compartmental system came with the finding that glutamine synthetase, the microsomal enzyme which converts glutamate to glutamine, is restricted to astrocytes [63, 72]. Over the past 50 years the concept of a "glutamate-glutamine cycle" has emerged as the central figuration in our understanding of brain amino acid metabolism [2, 24, 64–66, 80, 94, 95]. This model posits neuronal release of glutamate into the synapse, uptake into astrocytes via high-affinity transporters [79], glial amidation of glutamate to glutamine, export of the latter to neurons via specific transport systems [2, 53] and subsequent uptake of glutamine into neurons, where phosphate-dependent glutaminase regenerates glutamate in mitochondria.

Limitations of the Glutamate-Glutamine Cycle: the External Sources of Glutamate N

The essential elements of the glutamate-glutamine cycle have been integrated into a generally accepted conceptualization of brain amino acid handling. However, this model never was intended to be an exhaustive rendering of brain metabolism. Like all models, the glutamate-glutamine cycle is a deliberate simplification which highlights key elements of a complex system which become susceptible to experimental verification.

One limitation of the model is that it posits a stoichiometric relationship between glutamate released into the synapse, glutamine formed in astrocytes, and glutamate regenerated in neurons. However, McKenna [65] has emphasized that both neurons and astrocytes include multiple subcellular compartments of glutamate metabolism and that glutamate carbon derives from various sources, including glucose, lactate and 3-hydroxybutyrate. In addition, it is clear that astrocytes not only synthesize glutamine, they also oxidize this amino acid [19, 56, 66, 94, 99, 101]. Nor is all glutamine formed in glia restored to the neuronal compartment: transport of large neutral amino acids from blood-to-brain involves exchange with endogenously formed brain glutamine [18, 38, 51, 57].

A related conceptual problem of the glutamate-glutamine cycle is that it describes only intra-cerebral glutamate handling and ignores the crucial question of the precursors to glutamate in the peripheral circulation. Clearly, no organ system can function in isolation from blood. Oxidative metabolism inevitably give rise to end-products—primarily CO_2, H_2O and NH_3—which must be eliminated from the system. It also is probable that brain supports a net efflux of lactate [29, 85, 89], especially in response to heightened neuronal activi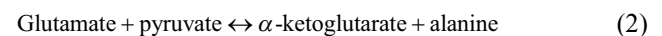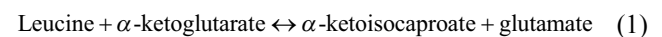ty. As noted above, a tonic export of glutamine from brain is essential to the uptake of neutral amino acids from blood [18, 38, 51, 57].

These losses from brain must be compensated. For individuals on a regular diet (that is, a non-ketogenic diet) the uptake of glucose from blood enables the replenishment of virtually all glutamate carbon [29, 84], but the precursors to glutamate N are less obvious. Potential donors include blood-borne glutamate, NH_3 or amino acids which donate an α-amino group via transamination. Direct importation of blood glutamate (usual concentration 20–50 μmoles/l) seems an infelicitous strategy, since trafficking of large amounts of glutamate through cerebral extracellular fluid invites the risk of evoking unbridled neuronal depolarization. Studies with $^{13}NH_3$, a positron-emitting species, demonstrate virtually no incorporation of ammonia N into glutamate via reductive amination of α-ketoglutarate [23]. Hence, transamination of amino acids transported from blood constitutes the primary mechanism for synthesis of glutamate. The transport studies of Smith et al. [87] showed that the brain uptake system for neutral amino acids is near saturation at physiologic blood concentrations and is approximately 50 % occupied by the sum of phenylalanine and leucine. It should be emphasized that this system is minimally (5 %) occupied by glutamine. Furthermore, at physiologic plasma concentrations, the predicted uptake of leucine exceeds that of any other amino acid [87].

Branched-Chain Amino Acids as a Source of Brain Glutamate N

BCAA are Major N Donor in Peripheral Tissues

A large body of evidence highlights the role of the branched-chain amino acids as nitrogen donors in peripheral tissues, particularly skeletal muscle, a major site for BCAA transamination [10, 42, 47, 62, 68]. Transamination of muscle leucine, derived both from degradation of skeletal muscle protein and from insulin-enhanced uptake of blood leucine, supports formation of glutamate, which, in turn, is transaminated to yield alanine:

$$\text{Leucine} + \alpha\text{-ketoglutarate} \leftrightarrow \alpha\text{-ketoisocaproate} + \text{glutamate} \quad (1)$$

$$\text{Glutamate} + \text{pyruvate} \leftrightarrow \alpha\text{-ketoglutarate} + \text{alanine} \quad (2)$$

The alanine is released to liver, where the carbon becomes a gluconeogenic substrate and the nitrogen is converted to urea [88]. These reactions assume heightened adaptive significance during starvation [35], when as much as 30 % of alanine N is derived from the branched-chain amino acids [45].

Uptake of BCAA into the CNS

Amino acid uptake into brain is an intense and active process [73] which obliges the coordinated action of several different transport systems. Influx of leucine into parietal cortex is faster (10 μmol/s/g wet weight) than that of any other amino acid. At physiologic blood concentrations of leucine (~50–150 μmol/l) transport is almost always saturated (Km 29 μmol/l) [87]. Amino acid uptake must be closely linked to transamination of these compounds, since the neutral amino acids are transported in exchange for intra-cerebral glutamine [18, 38].

After exiting blood, brain-bound metabolites enter astrocytes, which interpose between brain and vasculature [1, 12]. Uptake of neutral amino acids into cultured astrocytes is largely sodium-independent and enhanced by addition of glutamine to the medium [14, 15]; glial leucine transport is marked augmented (80%) by glutamine [14]. The likely Km for astrocyte leucine transport is 80–119 μmol/l.

Neurons also transport branched-chain amino acids via low- and high-affinity systems [76, 77, 92, 93, 99] which demonstrate marked sodium dependence [99]. Transport into synaptosomes is very rapid: Vmax of the low-affinity system is 62 nmol/min per mg protein and 118 nmol/min per mg protein for leucine and valine, respectively.

Handling of the Branched-Chain Amino Acids in the CNS

Metabolism of these amino acids within brain proceeds in three distinct steps, which are exemplified by the example of leucine:

1. Transamination: leucine + α-ketoglutarate ↔ α-ketoisocaproate + glutamate
2. Conversion of leucine-C into a form that enters the tricarboxylic acid cycle: α-ketoisocaproate →→ acetoacetyl-CoA → acetyl-CoA
3. Oxidation of leucine-C in the tricarboxylic acid cycle: acetyl-CoA →→ CO_2.

Complete oxidation of branched-chain carbon to CO_2 occurs in both brain slices and synaptosomes [20, 82, 83], but the rate of transamination greatly exceeds the rate of oxidation. Most branched-chain ketoacid formed in brain is converted back to the parent amino acid—in the case of α-ketoisocaproate to leucine [83]. This "reverse" reaction was at least twice the rate of α-ketoisocaproate oxidation in brain slices from young rats. Brand [11] demonstrated that in heart and kidney branched-chain ketoacids are primarily decarboxylated, but in brain these species are largely transaminated, in the process consuming an equimolar amount of glutamate.

It must be emphasized that many organs—and even cells within organs—do not oxidize branched-chain amino acids to completion. Instead, an intermediary metabolite may be released and then oxidized in another site. Indeed, as noted above, a fundamental motif of whole body amino acid metabolism involves release of branched-chain ketoacids from skeletal muscle and their subsequent oxidation in liver [35, 43]. This pattern even prevails within the brain. Thus, astrocytes can produce and release ketone bodies which they derive from the metabolism of leucine and fatty acids [3, 7, 41]. Brain also can oxidize ketone bodies [33, 66].

Transamination of Branched-Chain Amino Acids

Production of glutamate from BCAA obviously entails the transaminase (reaction 1; BCAT) noted above. Transamination of all three branched-chain amino acids occurs in both mitochondria and cytosol, with distinct enzymes identified in either compartment. The mitochondrial isozyme is a 41 kDa protein [48] while that in the cytosol is a 50 kDa species which likely forms a homodimer of apparent mass 91 kDa [40]. Distribution of the two transaminases, which share a ~58% homology [28], varies in a tissue-specific manner. Most tissues possess a mitochondrial activity, but in brain nearly 70% of activity is cytosolic [36, 46, 49, 90, 91]. Activity may vary broadly, even within a single organ: in rat kidney activity of the transaminase is 40-fold greater in the thick ascending limb of Henle than it is in the proximal convoluted tubule [16].

In rat brain the cytosolic transaminase is restricted to neurons [90, 91]. In many glutamatergic neurons this activity occurs in axons and nerve terminals, but in some GABA-ergic neurons activity predominates in the cell body. Autonomic neurons of the gut and axons of the sciatic nerve also contain primarily cytosolic activity. Astrocytes manifest primarily mitochondrial activity, but in developing oligodendroglia transamination also proceeds in the cytosol [8]. Hull et al. [46] found that in human brain the cytosolic enzyme is neuronal, but the mitochondrial variant is confined primarily to cells of the vascular endothelium. Both human glutamatergic and GABA-ergic neurons manifested the cytosolic enzyme in both cell bodies and axons.

The kinetic constants for the mitochondrial and cytosolic species are nearly identical [40]. Michaelis constants for the amino acids (1–5 mmol/l) exceed the estimated level of these compounds in brain (0.1–0.2 μmol/g wet weight) [86]. Similarly, the K_m for α-ketoglutarate (0.6 mmol/l) is greater than the brain level of this metabolite (0.2 μmmol/g wet weight) [32].

Studies of the kinetics, molecular expression or distribution of the transaminase are not a surrogate for direct measurements of flux from branched-chain amino acid N to the amino groups of glutamate and glutamine. The latter information obliges the use of an isotopic tracer. The positron-emitting species, ^{13}N, has been utilized productively to assay short-term transfer of nitrogen from ammonia to the amide and amino groups of glutamine [24], but the very brief half-life of the tracer (~10 min) precludes longer-term experiments or measurements at steady-state. To this end, we deployed [^{15}N]leucine (0.1 mmol/l) as a tracer in cultured astrocytes [98]. We utilized gas chromatography-mass spectrometry to assay the appearance of the label in [^{15}N]glutamate and [2-^{15}N]glutamine. In addition to the labeled leucine, the medium included 15 unlabelled amino acids and ^{15}NH$_4$Cl, thereby providing the cells with a panoply of alternative sources of N for glutamate synthesis from α-ketoglutarate. At steady-state the ratio of label of [^{15}N]glutamate/[^{15}N]leucine was 0.21, indicating that about one-fifth of glutamate N had been derived from leucine alone. This finding was congruent with an earlier study [96] in cerebellar explants in which we found that leucine and valine together contributed at least one-third of the amino groups utilized to form glutamate. A virtually identical result was obtained in cultures of GABA-ergic neurons [97].

Similar results have been obtained by investigators working in other systems. Thus, Zielke et al. [102] used microdialysis to perfuse leucine into the extracellular fluid of awake, freely-moving rats. They observed a significant increase in the concentration of interstitial large, neutral amino acids, suggesting that these compounds competed with leucine for uptake into brain cells via a common carrier. They also reported a significant increase in the level of interstitial glutamine, a finding consistent with the notion [14, 15] that: (a) transport of leucine and other neutral amino acids is achieved via exchange for internal glutamine and (b) that leucine N had been used for the purpose of glutamate synthesis. Kanamori et al. [55] used magnetic resonance spectroscopy to measure N transfer from leucine to glutamate in rats that received an intravenous infusion of [^{15}N]leucine. The authors estimated, assuming a K_m of brain branched-chain transaminase of 1.2 mM and a physiologic brain leucine concentration of 0.11 μmol/g, that leucine alone provided ~25 % of glutamate N.

Compartmentation of BCAA Metabolism in Brain: Possibility of a BCAA-Leucine Shuttle

It is the nature of glutamatergic (and GABA-ergic) neurotransmission to be at odds with the need to maintain metabolic stability of overall brain metabolism. Release of glutamate (or GABA) from nerve endings obliges the stoichiometric restoration of that which was lost. The straightforward import of fresh glutamate, conceivably from peripheral blood, would not be feasible, since this strategy would tend to elevate the external glutamate concentration, thereby rendering the system vulnerable to additional depolarization and both jeopardizing maintenance of an optimal signal-to-noise ratio and increasing the risk of excitotoxicity.

The glutamate-glutamine cycle (above) restores glutamate to neurons as glutamine, a non-neuroactive species which is present in abundance in brain extracellular fluid and which does not invite the risk of depolarization. This cycle is the essential figuration in our current understanding of brain amino acid handling, but, as would be true of any model, it is a simplified and incomplete rendering of a more complex biochemical and physiologic reality.

Several lines of evidence suggest that branched-chain amino acids and ketoacids also could be participants in cycle(s) which facilitate movement of N between astrocytes and neurons: (a) these compounds are swiftly transported into brain, thereby establishing a route of efficient articulation between brain and the peripheral circulation; (b) as noted above, the enzymes mediating transamination of branched-chain amino acids/ketoacids display a rigorous anatomic and subcellular (mitochondrial vs. cytosolic) distribution in neurons and astrocytes, suggesting the possibility of differential function in either cell type and/or organelle; (c) neither branched-chain amino acids nor the cognate ketoacids evoke neuronal depolarization, indicating that these compounds can be safely "trafficked" within the CNS; (d) transport systems for both these amino acids and the ketoacids are present on the surface of both neurons and astrocytes [13–15, 61, 76, 77, 92, 93, 99].

A structure for the proposed cycle is given in Fig. 1 (adapted from [26, 50, 59, 99, 100]). This model, which represents leucine as an exemplar of all three branched-chain amino acids, proposes that this compound enters brain in exchange for glutamine, the latter produced in astrocytes via glutamine synthetase. Astrocytes, the initial site of brain leucine metabolism, transaminate this amino acid to glutamate and α-ketoisocaproate (KIC) via mitochondrial branched-chain amino acid transaminase (BCATm). The glia then release the ketoacid to neurons, which take up this compound [61] and convert it back to glutamate [99] via cytosolic branched-chain transaminase (BCATc). This reaction results in consumption of neuronal glutamate. Leucine formed in neurons by "reverse" transamination then can be restored to astrocytes, thereby completing the cycle. As shown in Fig. 1, the putative "glutamate-BCAA cycle" (bottom of Fig. 1) would articulate with the "glutamine-glutamine cycle", with the latter furnishing the glutamine which supports brain entry of leucine via a counter-exchange mechanism (Fig. 1).
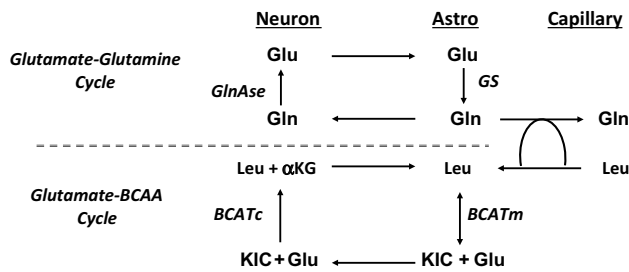
Fig. 1 Proposed schematic of glutamate-BCAA cycle. Leucine is an exemplar of the metabolism of isoleucine and valine. Leucine enters brain in exchange for glutamine (this exchange is symbolized in the figure by the arch which connects the glutamate-glutamine cycle and the proposed glutamate-BCAA cycle). Glutamine is formed in astrocytes via glutamine synthetase (GS). In astrocytes mitochondrial branched-chain transaminase (BCATm) forms glutamate and α-ketoisocaproate (KIC). Neurons take up this ketoacid, which is released by glia, and reaminate it to leucine via cytosolic branched-chain amino acid transaminase (BCATc). This transamination reaction consumes glutamate. Neurons release to astrocytes the leucine formed by branched-chain ketoacid reamination, thereby completing the cycle

Coupling of these two cycles would accomplish several purposes: (a) a mechanism for the importation of branched-chain amino acids into brain; (b) a possible mechanism for the "buffering" of neuronal glutamate when the concentration of the latter rises. Indeed, our prior work [99] suggested that in synaptosomes the "reverse" transamination pathway which results in formation of leucine is more active than the "forward" pathway which leads to the production of α-ketoisocaproate. This process might assume heightened physiologic significance during periods of relatively intense glutamate release since heightened neural activity recruits greater regional blood flow [74], in the process making available relatively more branched-chain amino acid to a set of neurons which must dispose swiftly of a relatively greater synaptic "load" of neuroactive glutamate. The putative glutamate-BCAA cycle (Fig. 1) would complement the glutamate-glutamine cycle by providing a mechanism for the transient buffering of synaptic glutamate when levels of this neurotransmitter abruptly rise.

We should, of course, avoid a facile extrapolation from the model implicit in Fig. 1 to physiologic reality. Brain is a heterogeneous organ in which metabolism may vary dramatically depending upon anatomic location and cell type. Thus, Bak et al. [4] found in cultured glutamatergic cerebellar neurons that valine—rather than leucine or isoleucine—was capable of maintaining neurotransmitter pools of glutamate in response to repeated depolarization.

Additional research will be needed to validate or refute this model. However, many features of this conceptualization are supported our current understanding of brain chemistry and physiology: (a) leucine and the other branched-chain amino acids have long been known to function as N donors in periph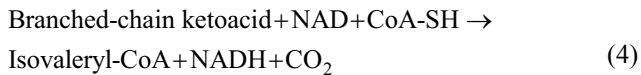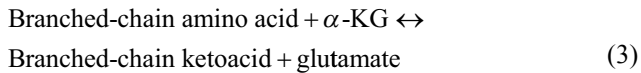eral tissues [35]. It would be unsurprising if a similar pattern exists in the CNS. (b) Brain actively transports these compounds: leucine enters brain more quickly than any other amino acid [87]. (c) Brain has abundant capacity for the transamination of branched-chain amino acids. Indeed, the brain is virtually unique among body organs in possessing both mitochondrial and cytosolic forms of branched-chain amino acid transaminase. (d) The rate of brain transamination of branched-chain amino acids greatly exceeds the rate of their oxidation [83], indicating that brain has ready access to a steady supply of the ketocids. (e) Neurons are capable of transporting branched-chain ketoacids [61]. (f) Transamination can proceed in either the "forward" or "reverse" direction, depending upon the relative concentration of the reactants. However, some evidence suggests [99] that in neurons the equilibrium favors reamination of the ketoacid to yield the parent amino acid. It seems unsurprising that reamination should prevail, given the fact that the intracellular concentration of the brain metabolic pool of glutamate is as high as 10–13 mmol/l [39], particularly in the neuronal compartment. Studies with a microdialysis system in the non-anesthetized rat demonstrated that infusion of α-ketoisocaproate into the extracellular fluid enhanced the oxidation of ^{14}C-glutamate and ^{14}C-glutamine [103], thus indicating active transamination of the branched-chain ketoacid to α-ketoglutarate, which was oxidized to CO_2 in the tricarboxylic acid cycle. (g) Studies with magnetic resonance spectroscopy suggest that carriage of leucine from neuron-to-astrocyte might suffice to replenish glial nitrogen pools [80], although this methodology may not render with adequate temporal or anatomic sensitivity the dynamic and fine-scale metabolic adjustments that undoubtedly occur in response to altered neuronal activity.

Brain Handling of Branched-Chain Amino Acids: Clinical Considerations

The foregoing review explored the role of the branched-chain amino acids in modulating brain handling of glutamic acid, an assignment which is pivotal to all cerebral metabolism. We encountered an extremely intricate system which obliges stringent anatomic segregation of enzymes into distinct cell types and even subcellular organelles, the polarization of transport processes to enable vectorality of metabolite transfer and, above all, the elaboration of control mechanisms which assure reasonable constancy of glutamate metabolism-even in a system characterized by episodic release of this neurotransmitter and the requirement to swiftly restore it to a neuronal compartment.

Clearly, an inherited mutation which affects the function of this tightly controlled system would be expected to have unwelcome and potentially severe consequences for brain metabolism. This hypothesis has been lamentably proven

accurate by our experience with maple syrup urine disease, or a deficiency of branched-chain ketoacid dehydrogenase (reaction 4, below):

$$\text{Branched-chain amino acid} + \alpha\text{-KG} \leftrightarrow$$
$$\text{Branched-chain ketoacid} + \text{glutamate} \qquad (3)$$

$$\text{Branched-chain ketoacid} + \text{NAD} + \text{CoA-SH} \rightarrow$$
$$\text{Isovaleryl-CoA} + \text{NADH} + CO_2 \qquad (4)$$

An inherited deficiency of branched-chain ketoacid dehydrogenase gives rise to maple syrup urine disease (MSUD) [17, 104]. The defect in this enzyme, which belongs to the same protein "family" as α-ketoglutarate dehydrogenase and pyruvate dehydrogenase, results in extreme accumulation in blood and tissues of branched-chain ketoacids as well as the parent amino acids, the latter generated via re-amination. Effective treatment includes a special diet which is purposefully low in the offending amino acids. If therapy can be instituted quickly—and all states today screen neonates for this disorder—severe brain damage may be avoidable. Unfortunately, metabolic decompensation can occur at any time, especially in association with the catabolic stress of acute infection. The affected child manifests lethargy, confusion, coma and, in severe cases, frank brain swelling [78]. Liver transplantation may ward off acute metabolic decompensation, but long-range followup of patients indicates that even such drastic intervention will not prevent eventual neurologic and psychiatric deterioration [69].

The cause of brain damage in MSUD is multi-factorial, but evidence suggests that the very factors which have made branched-chain amino acids vital contributors to healthy neurologic function become destructive and toxic agents if these amino acids and ketoacids are present in excessive amount. Thus, as noted above, the branched-chain amino acids, which brain so avidly transports [87], in high concentration deplete levels of other large neutral amino acids [30, 75] by efficiently excluding these metabolites from binding to the relevant transporter(s). The result is a failure of both brain protein synthesis and of neurotransmitter synthesis, since the latter—notably serotonin and dopamine—are formed from tryptophan and tyrosine.

Similarly, during a MSUD-induced crisis the brain concentrations of glutamate, glutamine and aspartate are very low—and correlated with the intensity of branched-chain amino acid accumulation. The cause appears to be a relative intensification of glutamate consumption via "reversal" of branched-chain amino acid transamination in response to an extravagant increase of the cognate ketoacids [30, 75]. It is thought that depletion of brain glutamate and aspartate impairs function of the malate-aspartate shuttle, the main mechanism in brain for the transfer of glycolytically-derived reducing equivalents from cytosolic to mitochondrion [60].

These pathologic events result in an increased level of both brain lactate and branched-chain amino acids—phenomena which have been observed with magnetic resonance spectroscopy during acute metabolic decompensation [52].

The foregoing analysis involves a profound—and often life-threatening—biochemical derangement which accompanies a near-complete inherited deficiency of branched-chain ketoacid dehydrogenase. In recent years, however, considerable research has focused on the possibility that less onerous increases of branched-chain amino acids and ketoacids might prove beneficial in preventing brain injury, possibly by subtly modulating intra-cerebral levels of glutamate and/or glutamine. To this end, investigators have scrutinized the therapeutic potential of dietary branched-chain amino acid supplementation in diverse pathologic conditions, among them hepatic encephalopathy [5, 27], fatigue [21, 37, 71], traumatic brain injury [22]; appetite and energy sensing [81], obesity [31] and epilepsy [34]. It is probable that future investigation will broaden this repertoire still more.

Conclusions

Branched-chain amino acids have emerged as "key players" in brain biochemistry. They serve as an important external source of amino groups for the synthesis of brain glutamate and, as branched-chain ketoacids, they may provide a mechanism for the "buffering" of internal levels of glutamate should the latter rise to potentially toxic concentration. The ketoacids may seem unlikely candidates for the latter role, since brain glutamate normally is present at a concentration (10–13 mmol/l) which exceeds that of branched-chain amino acids and ketoacids by orders of magnitude. However, it is important to emphasize that the glutamate pool which must be "buffered" is not the internal metabolic pool of neurons but the pool of neuroactive glutamate which is resident in the synapse. Obviously, intra-synaptic levels of glutamate—even after depolarization and release from nerve endings—are far lower than the internal concentration in neurons or astrocytes. Indeed, it is probable that the intra-synaptic concentration of glutamate is comparable to synaptic levels of branched-chain amino acids and ketoacids, which then could serve as "transamination partners" for glutamate.

It should be emphasized that branched-chain amino acids and/or ketoacids could prove therapeutically beneficial through mechanisms that do not involve their direct involvement in metabolic pathways. Leucine functions as a regulator of metabolism through its role as an activator of the mTOR signaling pathway [54, 58, 70]. Indeed, it may be that tissue damage in inborn errors of metabolism can result from derangements of leucine concentration [9]. Nonetheless, much evidence points to a salient role for

branched-chain amino acids and ketoacids as key players in the metabolism of the nervous system. Such a role would accord well with the well-established function of these compounds as essential pivotal carriers and exchangers in the nitrogen economy of the periphery.

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