COMMENTARY

The case for regulating indispensable amino acid metabolism: the branched-chain α-keto acid dehydrogenase kinase-knockout mouse

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BCAAs (branched-chain amino acids) are indispensable (essential) amino acids that are required for body protein synthesis. Indispensable amino acids cannot be synthesized by the body and must be acquired from the diet. The BCAA leucine provides hormone-like signals to tissues such as skeletal muscle, indicating overall nutrient sufficiency. BCAA metabolism provides an important transport system to move nitrogen throughout the body for the synthesis of dispensable (non-essential) amino acids, including the neurotransmitter glutamate in the central nervous system. BCAA metabolism is tightly regulated to maintain levels high enough to support these important functions, but at the same time excesses are prevented via stimulation of irreversible disposal pathways. It is well known from inborn errors of BCAA metabolism that dysregulation of the BCAA catabolic pathways that leads to excess BCAAs and their α-keto acid metabolites results in neural dysfunction. In this issue of the *Biochemical Journal*, Joshi and colleagues have disrupted the murine BDK (branched-chain α-keto acid dehydrogenase kinase) gene. This enzyme serves as the brake on BCAA catabolism. The impaired growth and neurological abnormalities observed in this animal show conclusively the importance of tight regulation of indispensable amino acid metabolism.

Key words: branched-chain amino acid, branched-chain α-keto acid dehydrogenase kinase (BDK), epilepsy, indispensable amino acid (essential amino acid), protein synthesis.

Twenty amino acids are required for protein synthesis. In humans and mammals, nine amino acids cannot be synthesized endogenously and/or in sufficient amounts. These amino acids, including the BCAAs (branched-chain amino acids) leucine, isoleucine and valine, must be acquired in the diet and are classified as nutritionally indispensable (essential) amino acids. If a single indispensable amino acid is limiting, protein synthesis is inhibited. The need of the organism for each indispensable amino acid for protein synthesis and for non-protein functions defines the individual daily indispensable amino acid requirement.

To balance the body's needs for BCAAs with the supply of BCAAs from the diet, the BCAA catabolic pathway is regulated. As a result, BCAAs are cleared efficiently when dietary intake is in excess of the body's needs and conserved when dietary intake of these amino acids is inadequate. The study by Joshi et al. [1] that appears in this issue of the *Biochemical Journal* shows there are physiological consequences of unregulated BCAA catabolism.

The first step in BCAA catabolism is transamination catalysed by BCAT (branched-chain aminotransferase) isoenzymes. There are two mammalian BCATs: a mitochondrial (BCATm) and a cytosolic (BCATc) isoenzyme [2]. In this reaction, nitrogen is transferred from a BCAA to α-ketoglutarate to form glutamate and the respective BCKA (branched-chain α-keto acid). Thus transamination provides the mechanism for dispersing BCAA nitrogen. On the other hand, transamination is a reversible reaction. In the cellular environment, most aminotransferases operate at substrate concentrations below the K_m (low-affinity high-capacity systems). So the forward reaction (net nitrogen transfer from BCAAs to glutamate) occurs when the keto acid products are irreversibly oxidized by the second enzyme in the catabolic pathway, the mitochondrial BCKDH (BCKA dehydrogenase) enzyme complex.

The BCKDH complex catalyses the oxidative decarboxylation of the BCKAs to form their respective branched-chain acyl-CoA derivatives and NADH. The mammalian BCKDH complex contains multiple copies of three enzymes [3]: a branched-chain α-keto acid decarboxylase (E1), a dihydrolipoyl transacylase (E2) and a dihydrolipoyl dehydrogenase (E3). BCKDH activity is regulated by: (i) a highly specific 44 kDa BCKDH kinase (BDK) that can reversibly associate and dissociate from the complex [4], and (ii) product inhibition. The kinase phosphorylates and inactivates the E1 enzyme. Kinase activity is regulated by changes in levels of the α-keto acid of leucine, α-keto-isocaproate, which inhibits the kinase, and changes in BDK gene expression in liver [4].

Joshi et al. [1] have disrupted the murine BDK gene thus generating a mouse without the enzyme that covalently inactivates the BCKDH complex. In the BDK$^{-/-}$ mouse, the BCKDH complex is constitutively fully active in all tissues. Therefore BCCA oxidation is only influenced by substrate supply (dietary BCAA content and the transamination reaction) or product inhibition (levels of NADH and branched-chain acyl-CoAs). The impaired growth observed in the offspring and amelioration of much of the growth retardation by feeding a high-protein diet suggests that without the covalent brake on BCAA oxidation these amino acids can become limiting for protein synthesis when the demand is high, i.e. during growth. Thus the observed growth impairment in BDK$^{-/-}$ animals shows the need for a sufficient supply of these essential amino acids and, by extrapolation, dietary protein in young animals.

A unique feature of BCAA metabolism is that, rather than being restricted to the liver as for most indispensable amino acids, the BCAA catabolic enzymes are distributed widely in body tissues, including the central nervous system [2]. With one exception

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(BCATc in neurons), all enzymes in the catabolic pathway are located in the mitochondria of the cell. The activity state of BCKDH ranges from > 90 % active in liver (low BDK activity), to intermediate in heart, kidney and brain, and to essentially inactive in skeletal muscle (high BDK activity) [5]. Because BCAT is not expressed in rodent liver, BCAAs ingested during a meal escape first-pass metabolism in the liver and are thus available to peripheral tissues and brain. The differential tissue- and cell-specific expression of the BCATs and BCKDH results in significant inter- and intra-organ exchange of BCAAs and their α-keto acid metabolites. Liver is thought to be the primary site of BCKA oxidation.

Without BDK, extrahepatic oxidation of BCAAs is not regulated, and it is likely that the site of oxidation shifts from liver to tissues that contain high levels of BCATm and BCKD, such as pancreas, skeletal muscle, heart, kidney [5] and brain, which contains both BCATs. It is thought that BCAAs are nitrogen donors for moving nitrogen in the form of alanine and glutamine from muscle amino acid oxidation to the liver for urea synthesis. They are nitrogen donors for synthesis of the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA (γ-aminobutyric acid) in brain [6]. This leads to the potential for excess synthesis of glutamate (α-ketoglutarate depletion), alanine and glutamine in tissues outside the liver. Thus further study of this mouse model may help us to better understand the specialized functions of BCAAs in body and central nervous system nitrogen metabolism.

Another feature of BCAAs is the role of leucine as an anabolic nutrient signal. Leucine communicates the presence of an ingested protein-containing meal to peripheral tissues. It stimulates insulin secretion by the β-cells of the pancreas [7] and protein synthesis [8] in skeletal muscle, adipose tissue and heart. Leucine stimulates protein synthesis in skeletal muscle through both insulin-dependent and -independent mechanisms [8]. The insulin-dependent mechanism is associated with signalling through the mTOR (mammalian target of rapamycin) pathway to 4E-BP1 (eukaryotic initiation factor 4E-binding protein) and ribosomal protein S6K1 (S6 kinase 1), whereas the insulin-independent effect is mediated by an unknown mechanism that may involve phosphorylation of eIF (eukaryotic translation initiation factor) 4G and/or its association with eIF4E. Leucine does not appear to affect the Met-tRNAi binding step in translation initiation and the phosphorylation status of eIF2 [8].

In the BDK-deficient mouse, it is likely that peripheral tissues still 'see' the meal-induced rise in plasma leucine; however, unrestricted oxidation would be expected to lower plasma and tissue BCAAs rapidly, perhaps prematurely turning off the leucine signal. Although fed plasma insulin and BCAA concentrations were not reported by Joshi et al. [1], the smaller size of many of the organs and tissues of the BDK$^{-/-}$ animals is consistent with decreased protein accretion as a result of BCAA limitation and/or reduced leucine signalling. As part of the leucine effect on protein synthesis is insulin-dependent, there is the possibility that changes in insulin sensitivity and/or secretion may contribute to the BDK$^{-/-}$ mouse growth phenotype. Also, insulin stimulation of amino acid uptake into skeletal muscle and other leucine target tissues provides the amino acid substrates for the increase in protein synthesis in response to a meal.

Most of the reported phenotype of the BDK$^{-/-}$ mouse is consistent with the hypothesis that the phenotype results from the decreased availability of BCAAs for protein synthesis and/or reduced leucine stimulation of protein synthesis. The intriguing neurological phenotype includes motor dysfunction and seizures (in the adult animals). Seizures can occur in patients with MSUD (maple syrup urine disease), which results from mutations in one or more proteins in the BCKDH complex (blocked BCAA oxidation) [3]. Unlike the BDK-deficient mouse, which exhibits lowered brain BCAA concentrations (presumably BCKAs as well), MSUD is characterized by elevated concentrations of plasma and tissue BCAAs and BCKAs. Although the molecular basis for the neurological effects of MSUD is not known, toxic effects are correlated with the rise in BCAAs and BCKAs. It would appear that low BCAA concentrations, resulting from a chronic limited supply due to excessive oxidation, have equally serious consequences for the central nervous system. The authors [1] measured the phosphorylation status of brain eIF2α and determined that its phosphorylation was higher in the BDK-deficient mouse brain at 3 weeks (seizures occur in adults), suggesting impaired global translation. The authors speculate that epilepsy results from reduced translation of as yet unidentified proteins that occurs during fetal and/or postnatal brain development.

Leucine does not directly affect eIF2α phosphorylation, but eIF2α phosphorylation status is affected by indispensable amino acid deficiency [9]. Select neurons in the piriform cortex respond to indispensable amino acid deficiency through sensing of the uncharged tRNA and activation of GCN2 (GC non-derepressing 2 kinase), which leads to increased phosphorylation of eIF2α. Activation of these neurons transmits the signal to the lateral hypothalamus, and the learned behaviour probably involves amygdaloid and hippocampal cells [9]. Glutamate is the transmitter of the primary output cells of the anterior piriform cortex. Thus it is possible that the chronic BCAA deficiency during development (especially during the postnatal period) influences strength and/or specificity in neural circuits that affect seizure susceptibility and motor function. It is also possible that unrestrained transamination of BCAAs in brain may alter the levels of excitatory glutamate and/or inhibitory GABA in populations of neurons. The BCATc isoenzyme is found in GABAergic and glutamatergic neurons [10]. This might create an imbalance between glutamate and GABA synthesis or result in depletion of α-ketoglutarate. α-Ketoglutarate is the substrate for glutamate synthesis, but it is also a citric acid cycle (tricarboxylic acid cycle) intermediate and required for oxidative energy production.

In conclusion, although it is well known that inborn errors of amino acid metabolism frequently have neurological consequences, the study by Joshi et al. [1] is the first to show that unregulated metabolism of BCAAs affects the nervous system. What is the brain pathology of the adult mouse, and is the phenotype the result of effects on protein translation and/or altered neurotransmitter metabolism? We wait expectantly to learn more about this intriguing animal model.

REFERENCES

1   Joshi, M. A., Jeoung, N. H., Obayashi, M., Hattab, E. M., Brocken, E. G., Liechty, E. A., Kubek, M. J., Vattem, K. M., Wek, R. C. and Harris, R. A. (2006) Impaired growth and neurological abnormalities in branched-chain α-keto acid dehydrogenase kinase-deficient mice. Biochem. J. **400**, 153–162

2   Hutson, S. M., Sweatt, A. J. and Lanoue, K. F. (2005) Branched-chain amino acid metabolism: implications for establishing safe intakes. J. Nutr. **135**, 1557S–1564S

3   Harris, R. A., Zhang, B., Goodwin, G. W., Kuntz, M. J., Shimomura, Y., Rougraff, P., Dexter, P., Zhao, Y., Gibson, R. and Crabb, D. W. (1990) Regulation of branched-chain α-ketoacid dehydrogenase and elucidation of a molecular basis for maple syrup urine disease. Adv. Enzyme Regul. **30**, 245–263

4   Shimomura, Y., Obayashi, M., Murakami, T. and Harris, R. A. (2001) Regulation of branched-chain amino acid catabolism: nutritional and hormonal regulation of activity and expression of the branched-chain α-keto acid dehydrogenase kinase. Curr. Opin. Clin. Nutr. Metab. Care **4**, 419–423

5   Suryawan, A., Hawes, J. W., Harris, R. A., Shimomura, Y., Jenkins, A. E. and Hutson, S. M. (1998) A molecular model of human branched-chain amino acid metabolism. Am. J. Clin. Nutr. **68**, 72–81

6   Yudkoff, M., Daikhin, Y., Grunstein, L., Nissim, I., Stern, J. and Pleasure, D. (1996) Astrocyte leucine metabolism: significance of branched-chain amino acid transamination. J. Neurochem. **66**, 378–385

7   Malaisse, W. J., Malaisse-Lagae, F. and Sener, A. (1984) The stimulus-secretion coupling of amino acid-induced insulin release metabolic interaction of L-asparagine and L-leucine in pancreatic islets. Biochim. Biophys. Acta **797**, 194–202

8   Kimball, S. R. and Jefferson, L. S. (2006) New functions for amino acids: effects on gene transcription and translation. Am. J. Clin. Nutr. **83**, 500S–507S

9   Gietzen, D. W. and Rogers, Q. R. (2006) Nutritional homeostasis and indispensable amino acid sensing: a new solution to an old puzzle. Trends Neurosci. **29**, 91–99

10   Garcia-Espinosa, M. A., Wallin, R., Hutson, S. M. and Sweatt, A. (2006) Widespread neuronal expression of branched-chain aminotransferase in the CNS: implications for leucine/glutamate metabolism and for signaling by amino acids. J. Neurochem., in the press