INDUCTION OF EXPRESSION OF BRANCHED-CHAIN AMINOTRANSFERASE AND ALPHA-KETO ACID DEHYDROGENASE IN RAT TISSUES DURING LACTATION

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1. ABSTRACT

This study was designed to determine the effect of lactation and weaning on the gene expression of branched-chain aminotransaminase (BCAT) and branched-chain α-keto acid dehydrogenase (BCKD) in different tissues of the lactating rat. BCAT activity increased in mammary tissue during lactation and was 6-fold higher than in virgin rats. This increase was associated with an increase in protein levels measured by immunoblot analysis, and with an increase in BCAT mitochondrial (BCATm) mRNA concentration. Twenty-four hours after weaning, BCAT activity, protein concentration, and mRNA levels in the dam decreased. BCAT activity, protein enzyme levels, and BCATm mRNA concentration in muscle were higher in weaning rats than in lactating rats. BCAT cytosolic (BCATc) mRNA was not expressed in mammary tissue, and there was no BCATc enzyme detected by Western blot in any physiological state. Mammary tissue BCKD activity increased and was active (dephosphorylated) during the lactation period. The level of enzyme also increased and the mRNA...
level for the E2 subunit in mammary tissue was 10-fold higher than the virgin values. Hepatic enzyme activity increased during weaning, and this was associated with the protein level and with the mRNA level of the E2 subunit. Muscle BCKD activity and protein content were the lowest of all tissues, and the E2 subunit mRNA level was barely detected by Northern blot analysis. The results suggest gene regulation of the two main catabolic enzymes of the branched-chain amino acid metabolism during lactation.

2. INTERORGAN METABOLISM OF THE BRANCHED-CHAIN AMINO ACIDS

The first step in the catabolism of nutritionally essential branched-chain amino acids (BCAA) leucine, valine, and isoleucine is a reversible transamination catalyzed by branched-chain amino acid transaminase (BCAT) (EC 2.6.1.42) (Ichihara & Koyama 1966) to produce their respective branched-chain α-keto acids: α-ketoisocaproate (KIC), α-ketoisovalerate (KIV) and α-keto-β-methylvalerate (KMV). The second and irreversible step in the catabolic pathway is catalyzed by the mitochondrial enzyme complex of the α-keto acid dehydrogenase (BCKD) (E1-EC 1.2.4.4; E2-no EC#; E3-EC 1.8.1.4). In this reaction the branched-chain α-keto acids are oxidatively decarboxylated to produce branched-chain acyl-CoA derivatives. Activity of the BCKD is regulated by phosphorylation/dephosphorylation, and the phosphorylation state of the complex is regulated primarily by the activity of the BCKD kinase (Paxton & Harris 1984).

In the rat, BCAT is not present in liver, but is found in most other organs and tissues, including skeletal muscle (Ichihara 1975; Hutson et al. 1978; Hall et al. 1993; Torres et al. 1993). Indeed, skeletal muscle is thought to be a major site of BCAA transamination (Harper et al. 1984). However, in muscle more than 95% of the BCKD enzyme complex is in the inactive form (Paxton et al. 1986). This limits the rate of muscle oxidation, conserving essential BCAA carbon, and is thought to result in significant reamination of BCAA in other tissues (Matthews et al. 1981). Oxidation appears to occur primarily in the liver where BCKD activity is highest. This rather elaborate metabolic scheme insures that significant increases in essential BCAA oxidation will occur only when BCAA accumulate in skeletal muscle.

An additional level of compartmentalization of BCAA metabolism occurs within the cell. Two isoenzyme forms of BCAT are found in mammals, mitochondrial (BCATm) and cytosolic (BCATc) (Ichihara 1975; Hall et al. 1993). BCATc is expressed only in brain, ovary, and placenta (Hall et al. 1993; Hutson et al. 1995). Unlike BCATc, the mitochondrial isoenzyme is expressed ubiquitously. Thus, BCATm must be the BCAT that is involved in the shuttling of branched-chain α-keto acid metabolites between skeletal muscle and other tissues (Harper et al. 1984; Lund & Williamson 1985). However, the precise function of BCATm (i.e., BCAA transamination) in tissues other than skeletal muscle has not been studied extensively. It has also been hypothesized that BCATm plays a significant role in body nitrogen metabolism (Harper et al. 1984).