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Developmental regulation of D-β-hydroxybutyrate dehydrogenase in rat liver and brain

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The activities of ketone-metabolizing enzymes in rat brain increase 3- to 5-fold during the suckling period before decreasing to the adult level after weaning. We have observed that a similar developmental pattern also exists for D-β-hydroxybutyrate dehydrogenase (BDH) in rat liver. Utilizing antibodies prepared against the purified protein we determined that the changes in BDH activities in both brain and liver are due to changes in the amount of BDH in the mitochondria. In vitro translations of isolated RNA followed by immunoprecipitation revealed that the increase in BDH activity and content was correlated with an increase in the level of functional BDH-mRNA in both liver and brain.

Hydroxybutyrate dehydrogenase, D-β-; Developmental regulation

1. INTRODUCTION

The metabolism of ketone bodies (D-β-hydroxybutyrate and acetoacetate) is carried out by three mitochondrial enzymes. D-β-Hydroxybutyrate dehydrogenase (BDH) catalyzes the conversion of D-β-hydroxybutyrate to acetoacetate [1]. This is followed by activation of acetoacetate by transfer of the CoA group from succinyl-CoA in a reaction catalyzed by 3-oxoacid-CoA transferase. Acetoacetyl-CoA is then cleaved by thiolase to yield two molecules of acetyl-CoA. Ketone bodies play an important role in energy metabolism and lipid biosynthesis in mammals [2–4]. D-β-Hydroxybutyrate dehydrogenase (BDH) has been shown to be subject to change under different conditions when ketone bodies are specifically needed [5–9]. There have been reports that the activity of brain BDH increases during the suckling period followed by a gradual decrease to the adult levels [10,11]. However, the basis for this transient increase in enzyme activity has not been determined. Using antibodies prepared against purified rat liver BDH, we have investigated the mechanism involved in this enzymatic change in liver and brain in developing rats. Our results show that the increased specific activity of BDH in liver and brain is due to an increase in the enzyme content, which is correlated with elevated levels of functional BDH-mRNA in these two tissues.

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Abbreviations: BDH, D-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30); BCA, bicinchoninic acid; BSA, bovine serum albumin; TCA, trichloroacetic acid; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

2.1. *Materials*

Chemicals are reagent grade unless otherwise specified. Solutions were prepared in deionized water. Goat anti-rabbit IgG-peroxidase conjugate, protein A-Sepharose CL-4B, 4-chloro-1-naphthol, and aprotinin were purchased from Sigma (St. Louis, MO). Guanidinium thiocyanate was from Fluka (Buffalo, NY). Adjuvant was from RIBI Inc. (Hamilton, MT). Nuclease-treated rabbit reticulocyte lysate was from Promega (Madison, WI). L-[^{35}S]Methionine (in vitro translation grade, 1115 Ci/mmol) and Enlightning rapid autoradiography enhancer were from DuPont-New England Nuclear (Boston, MA). Sprague-Dawley rats (3–40 days of age) were from a breeding colony maintained at the University of Alabama central animal facility.

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2.2. *Enzyme activity assays*

Liver mitochondria were isolated by the method of Bustamante et al. [12]. Brain mitochondria were prepared by the method of Russell and Patel [13]. Protein concentration was measured by the BCA protein assay [14] with bovine serum albumin (BSA) as protein standard. Mitochondrial membranes were disrupted by sonication of the mitochondrial suspension diluted in 10 mM Tris-HCl (pH 7.8), 1 mM EDTA with a Fisher Sonic Dismembrator Model 300. BDH activity was measured at room temperature as the rate of NAD reduction with D-β-hydroxybutyrate (sodium salt) as substrate as previously described [15].

2.3. *Immunocytochemical quantitation*

Rat liver BDH was purified to homogeneity using a modification of the procedure of Burnett and Khorona [16]. Polyclonal antibodies against the purified enzymes were raised in rabbits and the anti-BDH serum was prepared according to standard methods [17]. Immunochemical quantitation (Western blotting) of BDH in the mitochondria was done essentially as described [18,19].

2.4. *In vitro translation*

Liver and brain total RNA were prepared by the method of Chirgwin et al. [20]. RNA preparations were judged to be intact by agarose electrophoresis with visualization of the 18 S and 28 S ribosomal RNA bands [21]. The RNA was translated in vitro as described by Pelham and Jackson [22] using a micrococcal nuclease-treated rabbit reticulocyte lysate translation system incorporating L-[^{35}S]methionine at a concentration of 1.0 μCi/μl reaction volume.

2.5. *Immunoprecipitation*

Translation reactions were stopped by adding SDS to a final concentration of 4% (w/v) and boiled for 3 min in water bath. Products of translation were then diluted with an equal volume of phosphate-buffered saline (pH 7.2) followed by adding 4 vols of dilution buffer (2.5% Triton X-100, 190 mM NaCl, 60 mM Tris-HCl, pH 7.4, 6 mM EDTA, and 10 unit aprotinin). Aliquots were taken to determine TCA-precipitable counts. Immunoprecipitation was carried out as described by Anderson and Blobel [23].

2.6. *SDS-PAGE and fluorography*

SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli [24] in 0.1% SDS-Tris/glycine buffer on 10% separating gels. Following electrophoresis, gels were fixed in acetic acid/methanol/water (1:2:2, v/v) for 30 min and rocked in Enlightning rapid radioactive enhancer solution at room temperature for 30 min. Gels were then dried under vacuum at 60°C for 2–3 h. Fluorographs were prepared by exposing the dried gels to Kodak-Omat AR X-ray films at −70°C.

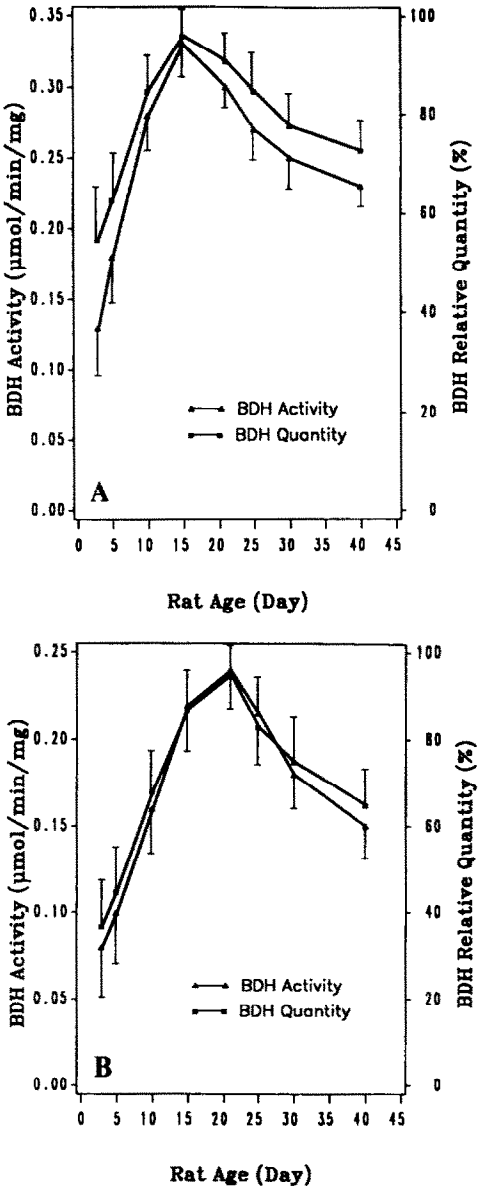

Fig.1. Developmental pattern of BDH in liver and brain in infant rats. Mitochondria were isolated from liver and brain tissues at different time points during development of young rats. BDH activity was assayed with sonicated mitochondria as previously described [15]. BDH content was measured by Western blotting of mitochondrial proteins fractionated on SDS-PAGE. The relative quantity of the enzyme is expressed as weight percentage determined by scanning of Western blots. Data are means ± SD (n = 3). A, liver BDH; B, brain BDH.

3. RESULTS AND DISCUSSION

Ketone bodies are important substrates for both energy metabolism and lipid biosynthesis in the developing rat brain [3,25]. Early studies demonstrated that developing rats had an increase in the activities of the ketone-metabolizing enzymes in brain during the suckling period [10,26]. In the present report, the activity of BDH has been measured in mitochondria isolated from brain as

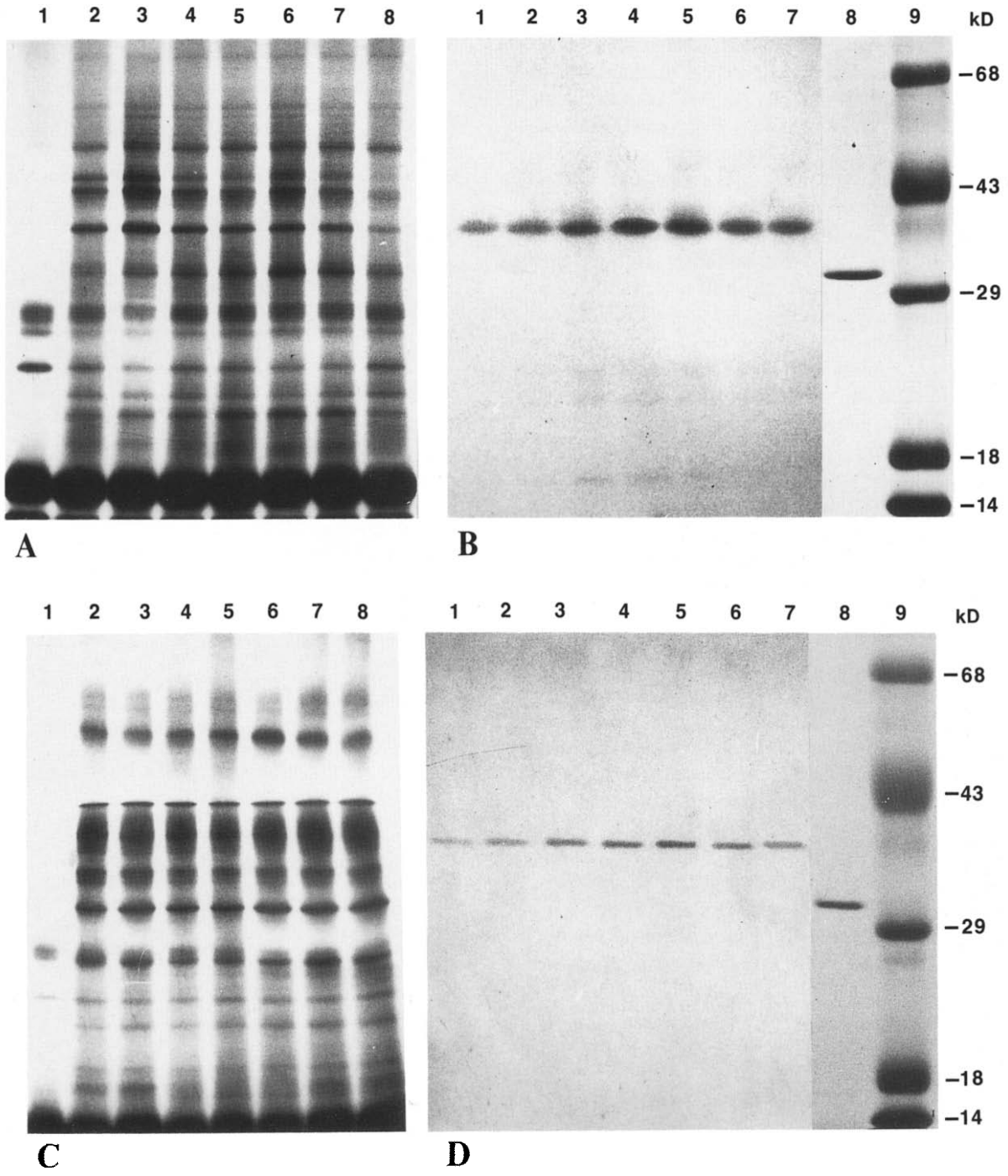

Fig.2. In vitro translation and immunoprecipitation. Total cellular RNA isolated at different time points from rat liver and brain tissues were translated in rabbit reticulocyte cell-free system with incorporation of L-[^{35}S]methionine at 30°C for 120 min. This was followed by immunoprecipitation, SDS-PAGE, and fluorography. A (liver) and C (brain) show the profiles of total translation products. Lane 1, no RNA added. Lanes 2–8, primed with RNA isolated on days 3, 5, 10, 15, 20, 30, and 40 from liver and brain, respectively. Immunoprecipitation of precursor BDH was shown in B (liver) and D (brain). Lanes 1–7, preBDH immunoprecipitated from the translation products shown in lanes 2–8 in A and C. Lanes 8 and 9, purified rat liver BDH and protein molecular markers stained with Coomassie blue.

well as liver at different times (days 3–40) during development. The enzyme activity has been determined to increase approximately 3-fold not only in brain tissue but also in liver (fig.1). The patterns of the activity changes in both liver and brain are similar, but slightly different in timing, i.e. the liver BDH activity increase precedes that of the brain enzyme by several days. This may reflect a sequential regulation or an induction effect, since the polarity of ketone metabolism is such that ketone bodies are produced in liver and utilized in nonhepatic tissues. The reason for the BDH activity increases has been attributed to an increase in the enzyme content by immunochemical quantitation. Our data show that the increase in BDH protein is well correlated with the results of a previous study, in which succinyl-CoA:acetoacetyl-CoA transferase, a key enzyme in ketone utilization, has been shown to increase in enzyme synthesis in developing rat brain [26].

To uncover the possible basis for the increase in BDH activity, we examined the level of functional BDH-mRNA by in vitro translation followed by immunoprecipitation. Equal amounts of total cellular RNA isolated from liver and brain at specific time points (days 3–40) were used to prime the in vivo translation. The synthesized precursor BDH (preBDH) was visualized on fluorography (fig.2). The increased preBDH synthesis from RNA sampled on days 15–20 represents the elevated levels of functional BDH-mRNA in both liver and brain tissues. The patterns of functional BDH-mRNA changes were shown to be similar to those of enzyme activity and content changes. This correlation suggests that the increase in the level of BDH-mRNA may be the basis for the increased specific activity of liver and brain BDH in developing rats.

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