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BRAIN RESEARCH

Research Report

β-hydroxybutyrate alters GABA-transaminase activity in cultured astrocytes

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ABSTRACT

The ketogenic diet has long been recognized as an effective treatment for medically refractory epilepsy. Despite nearly a century of use, the mechanisms underlying its clinical efficacy remain unknown. One of the proposed hypotheses for its anti-epileptic actions involves increased GABA concentration in the brain due to ketone bodies that become elevated with a ketogenic diet. In recent years, the notion that astrocytes could play a role in the evolution of abnormal cortical excitability in chronic neurological disorders, such as epilepsy, has received renewed attention. The present study examined the effects of β-hydroxybutyrate, a ketone body, on GABA metabolism in rat primary cultured astrocytes. When β-hydroxybutyrate was added to culture medium, GABA-transaminase (GABA-T) mRNA expression was significantly suppressed in time- and dose-dependent manners. GABA-T enzymatic activity in β-hydroxybutyrate-treated astrocytes was also suppressed, in accordance with its gene expression. These effects were evident after 3 days of culture, which might coincide with depleted intracellular glycogen. GABA transporter, GAT-1, gene expression was strongly suppressed in cultured astrocytes after 5 days of culture with β-hydroxybutyrate, although other type of GABA transporters did not display significant changes. These results suggest that β-hydroxybutyrate induced by ketogenic diet may increase GABA concentration in the epileptic brain by suppressing astrocytic GABA degradation, leading to antiepileptic effects.

1. Introduction

Glucose is the major energy source for cells in the central nervous system. However, under fasting condition or insufficient blood glucose utilization, ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone) are produced from fatty acids in the liver. The ketone bodies serve as an alternative energy source in extrahepatic tissues, particularly in the brain.

The ketogenic diets consist of high-fat, low-carbohydrate, and low-protein content, and have been used for patients with medically refractory epilepsy for nearly 90 years (Bough and Rho, 2007; Hartman et al., 2007). Although clinical efficacy of the ketogenic diet has been widely accepted, the biochemical basis for the anticonvulsant mechanisms has not been fully characterized. One probable explanation thought is that the energy source switches from glucose to

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fatty acids and ketone bodies may alter neurotransmitter concentrations, such as glutamate, aspartate, and γ-amino-butyric acid (GABA), which are involved in neuronal excitation in the brain. However, mechanisms underlying suppressive effects against seizure of ketone bodies remain unclear. Some studies reported that patients with elevated plasma ketone levels show better seizure-controls (Greene et al., 2003; Freeman et al., 2007), but other studies have failed to demonstrate association between the concentration of β-hydroxybutyrate and acetoacetate in plasma and seizure threshold (Bough et al., 1999). Thus the association between seizure-controls and plasma ketone levels has not been established yet.

GABA is a major inhibitory neurotransmitter in the brain. Fine-tuning and homeostatic balance of GABAergic inhibition in the brain is essential for many integrative brain functions. Down-regulation of the GABAergic system in the brain is associated with various neurological disorders, including epilepsy (Sperk et al., 2004; Treiman, 2001). Therefore, control of the GABAergic system has been a focus of epilepsy treatment. GABA concentrations in the brain are strictly controlled by three factors: glutamate decarboxylase (GAD65 and GAD67), GABA-transaminase (GABA-T), and GABA transporters (GATs) (Conti et al., 2004; Treiman, 2001). GAD synthesizes GABA from glutamate, whereas GABA-T catabolizes GABA to succinic semialdehyde. Furthermore, rapid termination of GABAergic activity is achieved by GABA reuptake through GATs into both neurons and astrocytes (Conti et al., 2004). In animal models of epilepsy, the ketogenic diet potently inhibits seizures evoked by GABAergic

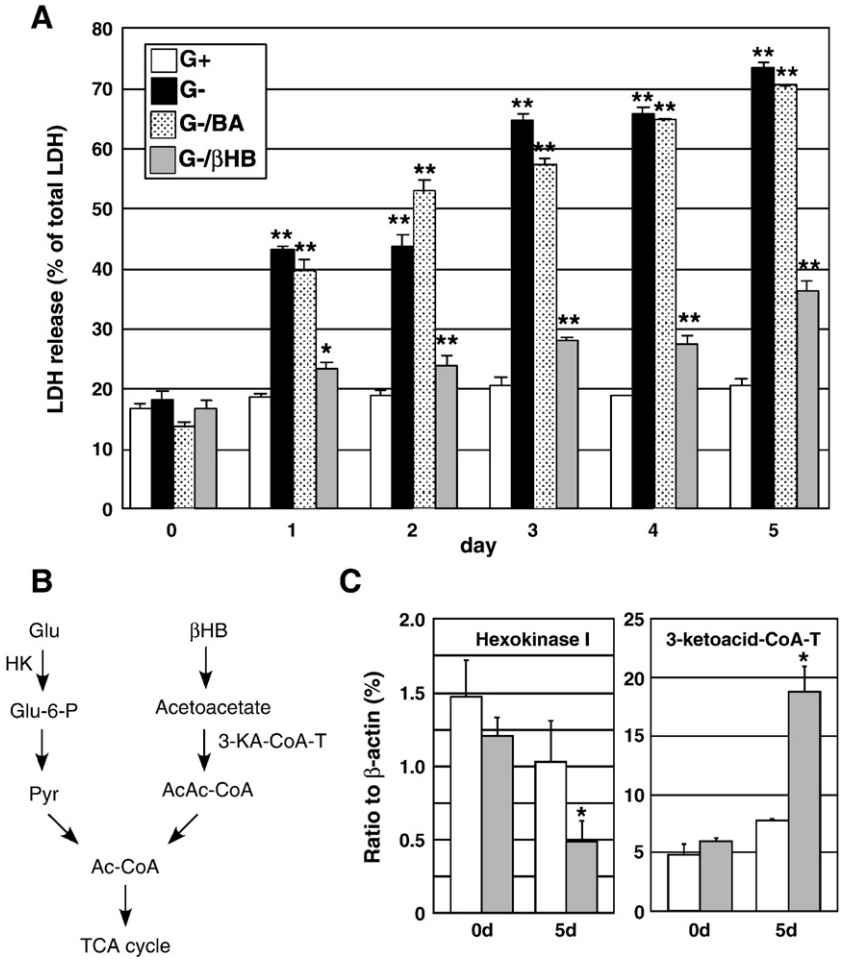

Fig. 1 – (A) Viability of cultured astrocytes under serum-free/glucose-free condition in the absence (G–) or presence (G–/βHB) of 10 mM β-hydroxybutyrate or 5 mM butyric acid for the indicated days was measured by LDH assay. Each column indicates a percentage (%) of released LDH, compared to total LDH. Values represent by mean ± SD from triplicate experiments and statistical analysis of G+ condition vs. other conditions at each culture day. *P < 0.01, **P < 0.001. (B) Scheme of metabolic pathway of glucose and ketone bodies. Glu, glucose; G-6-P, glucose-6-phosphate; Pyr, pyrvate; TCA cycle, tricarboxylic acid cycle; βHB, β-hydroxybutyrate; AcAc-CoA, acetoacetyl-CoA; Ac-CoA, acetyl-CoA; 3-KA-CoA-T, 3-ketoacid-CoA-transferase. (C) Astrocytes were cultured under serum-free/glucose-presence (G+) or serum-free/glucose-free conditions with 10 mM β-hydroxybutyrate (G–/βHB) for the indicated times. Total RNA was prepared from these cells, and the gene expression of the enzyme related to glycolysis and ketone metabolism was investigated by quantitative real-time RT-PCR. Each column indicates a ration to β-actin and values represent by mean ± SD from triplicate experiments. *P < 0.01 compared to G+ condition at each culture day.