β-Hydroxybutyrate Reverses Insulin-Induced Hypoglycemic Coma in Suckling–Weanling Mice Despite Low Blood and Brain Glucose Levels

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In normal suckling–weanling mice, DL-β-hydroxybutyrate (30 mmol/kg ip) stimulated insulin secretion and reduced plasma glucose levels. In the brains of these animals, glucose levels were tripled due to a reduced rate of glucose utilization (determined by deoxyglucose phosphorylation). Other metabolite changes were compatible with inhibition of hexokinase, phosphofructokinase, glyceroldehyde-P-dehydrogenase, and pyruvate dehydrogenase activities. In contrast to the decrease in cerebral glycolysis, metabolite changes were compatible with an increase in the Krebs citric acid metabolic flux. The brain energy charge was also elevated. While it is generally believed that ketone bodies cannot sustain normal brain metabolism and function in the absence of glucose, DL-β-hydroxybutyrate (20 or 30 mmol/kg ip) reversed insulin (100 U/kg sc)-induced hypoglycemia despite the persistence of a critically reduced plasma glucose concentration and near-zero brain glucose levels. Metabolic correlates of possible significance in the behavioral recovery from coma were reductions of the elevated levels of brain aspartate to below normal and ammonia levels to normal. Levels of acetyl CoA were unchanged both before and after treatment with β-hydroxybutyrate.

KEY WORDS: brain ketone-body metabolism; insulin hypoglycemia; β-hydroxybutyrate; brain carbohydrate, amino acid, ammonia, energy, and coenzyme A metabolism; cerebral rate of glucose utilization.

INTRODUCTION

In 1967 Owen and his collaborators found that obese adult patients who were starved for 5 to 6 weeks exhibited no deficits in cognitive or motor function and had normal electroencephalograms, despite the fact that the patients’ calculated sources of glucose were inadequate to provide energy for the brain for the whole period of

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starvation. What had happened was that ketone bodies had largely replaced glucose as the brain’s metabolic fuel; but glucose still accounted for about 30% of the oxygen consumed. Further, plasma glucose levels in these patients were relatively normal (4 to 5 mM). Since glucose at these concentrations tripled the rate of oxidation of β-hydroxybutyrate in rat brain slices (Ide et al., 1969), it was of interest to determine if β-hydroxybutyrate could support normal brain function with critically low levels (less than 1 mM) of glucose in plasma. To answer this question we examined the effect of β-hydroxybutyrate injection on selected aspects of brain carbohydrate, amino acid, coenzyme A, and energy metabolism in normal suckling-weanling mice and profoundly hypoglycemic littermates which had been injected with insulin.

**MATERIALS AND METHODS**

*Preparation of Animals*

Litters of normal suckling 19- to 21-day-old Swiss–Webster mice were used; the average weight was 12.5 ± 0.3 g (N = 60). (Animals of this age were used because of our clinical and research interests.) Litters were divided into four weight-matched groups: (1) normal mice injected with sterile 0.9% NaCl, (2) normal mice injected with β-hydroxybutyrate, (3) normal mice injected with insulin, and (4) hypoglycemic mice treated with β-hydroxybutyrate. Animals were treated as described below.

*Insulin Hypoglycemia.* Hypoglycemia was induced by the subcutaneous injection of 100 U/kg of crystalline zinc insulin (Iletin; Eli Lilly and Co., Indianapolis, Ind.) in a volume of 10 ml/kg; control littermates received an equivalent volume of 0.9% NaCl.

*β-Hydroxybutyrate Treatment.* Thirty milliliters per kilogram of 1 M DL-β-hydroxybutyrate (15 mmol/kg of the physiologic D enantiomorph) was injected intraperitoneally into normal control and insulin-injected mice when the latter exhibited signs of hypoglycemic stupor or coma (1.5 to 2 hr after insulin injection); other mice received 0.9% NaCl.

Twenty minutes after the last injection (0.9% NaCl or β-hydroxybutyrate), animals were decapitated, the head falling directly into liquid nitrogen, with constant stirring to facilitate rapid freezing of the small isolated head. Blood from the severed neck vessels was collected in heparinized capillary tubes (Caraway, Dade Division, American Hospital Supply Corp., Miami, Fla.). Frozen heads were stored at −85°C until the time of dissection.

*Cerebral Metabolic Rate for Glucose.* The effect of β-hydroxybutyrate injection on the rate of cerebral glucose utilization in normal mice was evaluated by a modification of the procedure of Crane et al. (1978). Fourteen minutes after intraperitoneal injection of 20 mmol/kg of sodium DL-β-hydroxybutyrate or 0.9% NaCl (controls), the mice were injected subcutaneously with 0.2 μCi/g body weight of 2-deoxy-D-[1-14C]glucose (14C-DOG), followed in 2 min by another subcutaneous injection of 0.5 μCi/g body weight of 2-deoxy-D-[1-3H]glucose (3H-DOG). Two minutes later (20 min after the injection of β-hydroxybutyrate or 0.9% NaCl) the animals were