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Protective effects of d-3-hydroxybutyrate and propionate during hypoglycemic coma: Clinical and biochemical insights from infant rats

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ABSTRACT

Background: D-3-hydroxybutyrate (3OHB) is an alternative energy substrate for the brain during hypoglycemia, especially during infancy. Supplementation of 3OHB during sustained hypoglycemia in rat pups delays onset of burst suppression coma, but is associated with white matter injury and increased mortality. The biochemical basis for this ambivalent effect is not known. It may be related to an anaplerotic or gluconeogenetic deficit of 3OHB.

Methods and results: We studied clinical alertness, EEG and brain metabolites (acyl-carnitines, amino acids, glycolytic and pentose phosphate intermediates) in 13 day-old rat pups during insulin induced hypoglycemic coma and after treatment with 3OHB alone or in combination with the anaplerotic substrate propionate. Clinically, treatment with 3OHB and propionate resulted in an alert state and EEG improvement, while treatment with 3OHB alone resulted in an improved EEG but animals remained clinically comatose. Biochemically, both treatments resulted in correction of cerebral glutamate and ammonia levels but not of gluconeogenetic substrates and pentose phosphate metabolites.

Conclusion: 3OHB treatment restores glutamate metabolism but cannot restore a glycolytic or pentose phosphate pathway deficit. Additional treatment with propionate significantly improved the clinical protective effect of 3OHB in hypoglycemic coma.

1. Introduction

Hypoglycemia is a frequent condition in neonates and infants. Supplementation of glucose to correct low blood glucose levels is the mainstay of treatment of acute hypoglycemia. Treatment of recurrent hypoglycemia is challenging and long term neurological sequelae like psychomotor deficits and epilepsy are difficult to avoid [1–3]. Hypoketotic hypoglycemia, which occurs in preterm infants and in hyperinsulinemic hypoglycemia, is considered particularly aggressive because ketone bodies (D-3-hydroxybutyrate (3OHB) and acetoacetate), which are metabolized to acetyl-CoA, are not available as alternative energy substrate.

Although ketone bodies mitigate short term effects of hypoglycemia [4–7] they cannot substitute entirely for glucose. We have recently shown that 3OHB supplementation increases the latency to hypoglycemic coma in rat pups. However the overall neuroprotective effect is limited, because the improved electrophysiological and clinical tolerance to hypoglycemia is associated with white matter injury and decreased survival after coma [8].

Biochemically the limited effectiveness of 3OHB during sustained hypoglycemia might be due to its inability to correct a deficiency of glycolytic substrates that are essential for anaplerosis and for the pentose phosphate pathway in the brain. Anaplerosis is particularly important, because the pool of citric acid cycle intermediates is the exclusive source of cerebral glutamate synthesis providing the basis for neurotransmitter metabolism and ammonia detoxification [9–11]. The pentose phosphate pathway supports oxidative defenses which are particularly important during states of energy deprivation and metabolic stress [12–14].

To further explore the effects of 3OHB on cerebral function and metabolism, we investigated clinical, electroencephalographic and biochemical effects of 3OHB treatment in insulin induced hypoglycemic rat pups. Considering the importance of cerebral anaplerosis, we also investigated changes after treatment with a combination of 3OHB and propionate which is a precursor of succinyl-CoA and serves as an alternative anaplerotic substrate (Fig. 1) [15–17].

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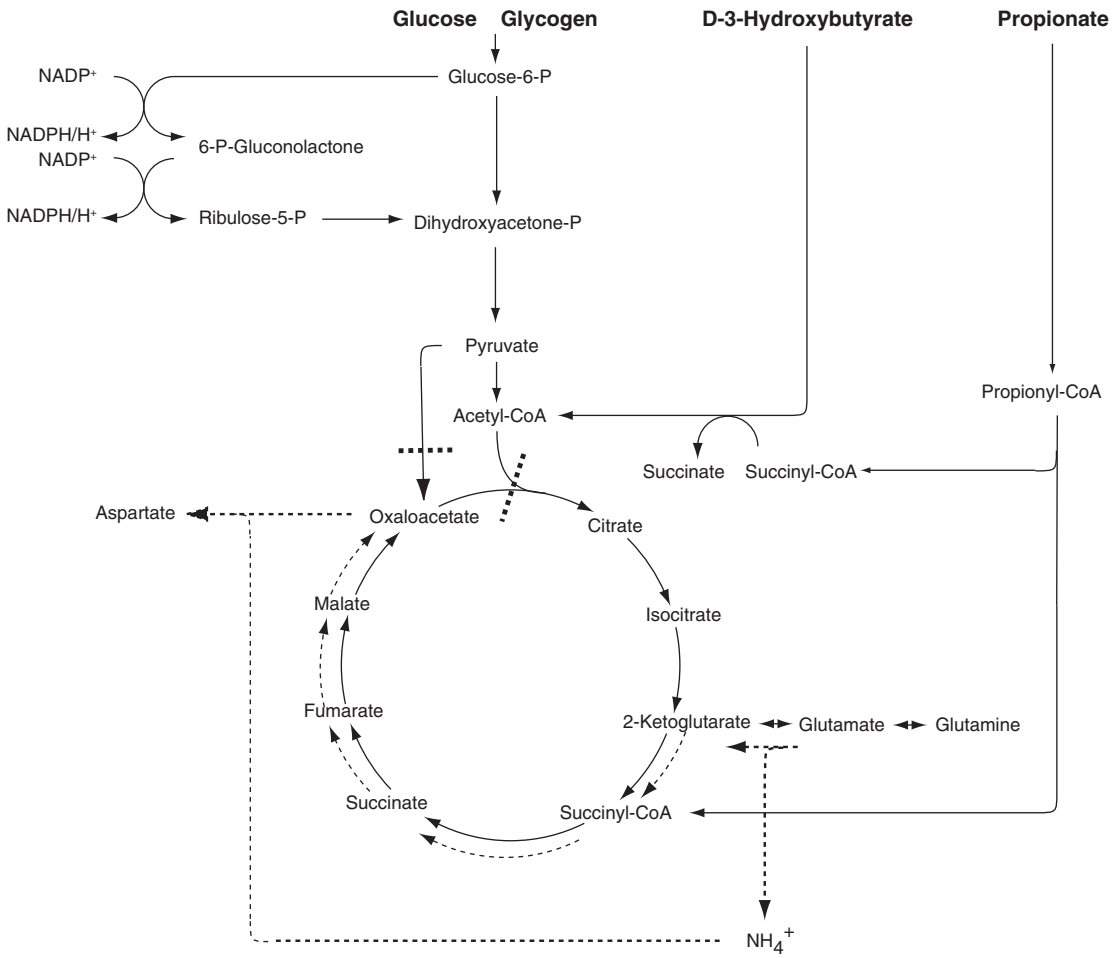

Fig. 1. Metabolic pathways for glucose, D-3-hydroxybutyrate, and propionate, schematically. Glycolysis generates acetyl-CoA for oxidation, NADPH for redox defenses (pentose phosphate pathway), and oxaloacetate for anaplerosis (pyruvate carboxylase pathway). 3OHB catabolism generates acetyl-CoA for oxidation only. Propionate is metabolized to succinyl-CoA and represents an alternative anaplerotic source. Note that succinyl-CoA is not only a citric acid cycle intermediate but is also required for the ketolytic activation of 3OHB. During hypoglycemia, glutamate is utilized as endogenous energy substrate (dashed lines). Following de- or transamination to alpha ketoglutarate, it enters the citric acid cycle and is converted to oxaloacetate. Glucose deprivation results in a shortage of acetyl-CoA, so that citric acid synthesis from oxaloacetate is blocked. Instead, oxaloacetate is aminated to aspartate (truncated citric acid cycle, [33]).

2. Materials and methods

2.1. Experimental setting

All experiments were performed according to the guidelines of the Canadian Council on Animal Care, and were approved by the Animal Care Committee of the University of British Columbia. The experimental setting was as previously described [8]. In brief, we used female Sprague Dawley rat pups on postnatal day (PND) 13. Age selection was based on age-specific EEG patterns during postnatal development in rats so that effective monitoring during hypoglycemia was feasible. EEG pattern and other parameters of cerebral maturation (enzymatic, synaptic) in PND 13 rats correspond to the early postnatal period in humans [18–23]. PND 13 rats are exclusively milk fed and have high cerebral ketone utilization rates [24]. Only female pups were used in order to standardize experimental conditions.

Hypoglycemia was induced and sustained by subcutaneous injections of 10 U/kg regular human insulin (Humulin R, Eli Lilly, Canada) at the start point of the experiment and at 120 min. We monitored heart rate and oxygen saturation by pulse oximetry and respiratory rates by observation. Atropine (1 mg/kg s.c.) was given 1 h after the first insulin dose to prevent bradycardia. We administered oxygen by facemask when respiratory rates were less than 30 min^{-1} to avoid hypoxia. For the clinical assessment of brain function we used an alertness score as previously described [8]. Briefly, this score evaluates voluntary movements, muscle tone, posture and reflexes. Scores range from 11 denoting alertness, to 0 denoting deep coma. For the assessment of electrical brain function we used electroencephalography [8]. For quantitative analysis of EEG suppression during the various stages of coma and as a measure of treatment response, we calculated the EEG suppression ratio (SR) online as previously described [8]. The SR is the ratio of the duration of suppression and the total duration of any given EEG epoch. It ranges from 0 (continuous EEG activity during an alert state) to 1 (completely suppressed EEG activity in severe coma).

2.2. Treatment protocol

After the induction of hypoglycemia, animals progressed clinically and electroencephalographically through characteristic stages to a deeply comatose state. Animals were treated when the EEG demonstrated a burst suppression pattern (burst suppression coma). At this stage, cerebral glycogen stores are largely exhausted [25,26] and are no longer a significant endogenous source for glycolysis. Animals were treated in three groups: 1) a single dose of D-3-hydroxybutyrate (3OHB), 2) a combined dose of D-3-hydroxybutyrate and propionate (3OHB + Prop), and 3) a single dose propionate (Prop). Treatment was given as