Effect of chronic administration of β-hydroxybutyrate in spontaneously epileptic kcna1-null mice measured with Manganese Enhanced MRI (MEMRI)

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Introduction

The ketogenic diet (KD) is now an established treatment for medically intractable epilepsy. Although the underlying mechanisms of KD action have yet to be fully elucidated, it is well known that the metabolic alterations accompanying this therapy mimic the physiological changes induced by fasting – wherein circulating glucose levels are decreased and ketones – notably, β-hydroxybutyrate (BHB), acetoacetate (ACA) and acetone – levels are elevated. Although several studies have shown direct acute anticonvulsant properties of ACA and acetone, it remains unclear whether BHB has similar activity. Recent studies have shown that the KD can suppress the mammalian target of rapamycin (mTOR) signaling pathway and reverse brain lesions in an experimental model of multiple sclerosis.[1, 2] Here we asked whether BHB alone can exert anticonvulsant activity in spontaneously epileptic *Kcna1*- null (knockout; KO) mice relative to KO mice treated with the full KD. We used Manganese Enhance MRI (MEMRI) to evaluate whether BHB's protective effects against seizures are associated with preservation of hippocampal integrity.

Methods

Animals: Spontaneously epileptic Kcna1-null (KO) mice were generated using heterozygous breeding pairs. Pups were weaned at P18 and genotyped by PCR analysis of tail genomic DNA. Experimental mice were matched for age, and at P21- 23, were treated with the KD and either standard diet (SD) or SD with subcutaneous administration of BHB through Alzet osmotic mini-pumps for 2-3 weeks. BHB measurement: Blood from tail clippings were used to measure BHB and glucose levels. Animals were monitored every 7 days at the same time using the Precision Xtra blood glucose and ketone monitoring system. Video-EEG study: EEG electrodes were implanted through parasaggital burr holes in either wild type (WT) mice or KO mice around P28. Following a 3-day recovery period, seizure activity was assessed over 72 continuous hours using wireless transmitter (DSI international) coupled to a Stellate video-EEG recording system. MRI imaging: After over 2 wks of BHB treatment, hippocampal structural changes were measured with MEMRI. T1-weighted images were acquired 24 hours after i.p. injection of 0.2 mM/kg $MnCl_2$ using a 7T Bruker Biospec MRI with a surface receive/volume transmit coil configureation (3D FLASH, TE=3.795ms, TR=15.0ms, NEX=4, α=35.0°, FOV= 25.6mmx25.6mmx25.6mm, Matrix=384x384x128). MRI data in hippocampus were analyzed using the MEDx3.4.3 software package (Medical Numerics, VA, USA) on a LINUX workstation.

Results

Figure 1A compares mean daily seizure frequencies over a 3-day recording period in saline treated KO mice on standard diet (SD), BHB- treated KO mice on SD and KD fed KO mice. Significant differences were found between mice on SD and BHB, while no difference was found between BHB and KD mice. Changes in MEMRI signal intensity showing the protective effect of BHB on seizure-induced structural hippocampal alterations are shown in Figure 1B and C. T1-weighted images were collected with a 7T MR scanner after 16-17 days of BHB administration. Saline-treated KO mice exhibited a reduction of signal intensities in whole hippocampal regions including dentate gyrus (DG), CA1 and CA3 subfields ($P<0.05$) when compared to WT. No significant differences were seen in DG between BHB-treated KO and saline-treated WT mice, while BHB treatment led to partial recovery of the MR signal in the CA1 and CA3 regions in KO mice.

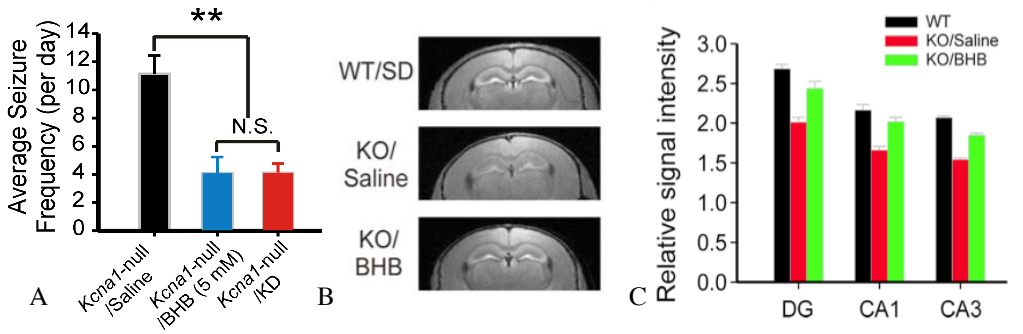

Figure 1:A) Significant difference in seizure frequency between saline treated and both KD and BHB treated mice. B) T1 weighted MEMRI images of WT, KO and BHB treated mice C) Reduced MEMRI contrast in hippocampus subfields with BHB treatment

Conclusion

The data presented here provide evidence for the functional protective link between the KD and its primary metabolic substrate, β-hydroxybutyrate, in spontaneously epileptic Kcna1-null mice. Increases in cellular bioenergetic reserves induced by either the KD or BHB may account for their anticonvulsant properties and preservation of hippocampal integrity in epileptic brain.[3,4]

References

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