Brain microvascular endothelial cells are sensitive to hypoglycemia, and partially rescued by ketone bodies

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INTRODUCTION

The blood-brain barrier (BBB) is a component of the neurovascular unit formed by specialized brain microvascular endothelial cells (BMECs) enclosed within a basement membrane and surrounded by astrocytes, pericytes and neurons.

Glucose represents the main source of energy of the CNS, as 20% of daily glucose intake is directed towards the brain. Glucose transport inside the CNS is occurring mostly via BBB in the presence of several glucose transporter isoforms (GLUTs). Although glucose metabolism has been mostly investigated in the lens of astrocyte-neurons axis, the fate of glucose and its metabolism at the BBB remains elusive.

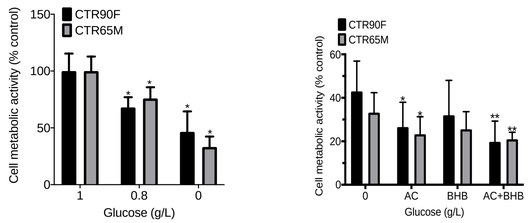
GLUT1 deficiency syndrome (GLUT1DS) is an autosomal dominant haploinsufficiency characterized by mutations in SLC2A1 resulting in impaired GLUT1 expression and/or activity. Patients suffering from GLUT1DS suffer from epileptic seizures, intellectual disabilities, and movement disorders.

The aim of the study is to investigate the effect of hypoglycemia and ketone bodies on the barrier function and glucose metabolism in vitro.

MATERIALS AND METHODS

Two iPSC lines (CTR90F and CTR65M), previously characterized by our group (Patel et al., J Neurochem 2017, Al-Ahmad, AJP Cell Physiol 2017) were used in the study. Cells were supplemented with ketone bodies (KB, 4microM beta-hydroxybutyrate and 1mM acetoacetate) in glucose deprived (0.8g/l) condition. Changes in GLUTs expression was assessed by immunofluorescence and flow cytometry. Change in glucose uptake was assessed using 14Cglucose and change in glycolytic flux using SeahorseXF24 flux analyzer. Quantitative analysis of beta hydroxybutyrate in hypoglycemic condition is assessed using liquid chromatography and mass spectrometry. Changes in barrier function was assessed by transendothelial electrical resistance (TEER), and fluorescein permeability

Figure 1: iPSC derived BMECs display differences in cell metabolic activity at different concentrations of glucose



RESULTS

Figure 2: iPSC derived BMECs display glucose dependent glucose transporter expression

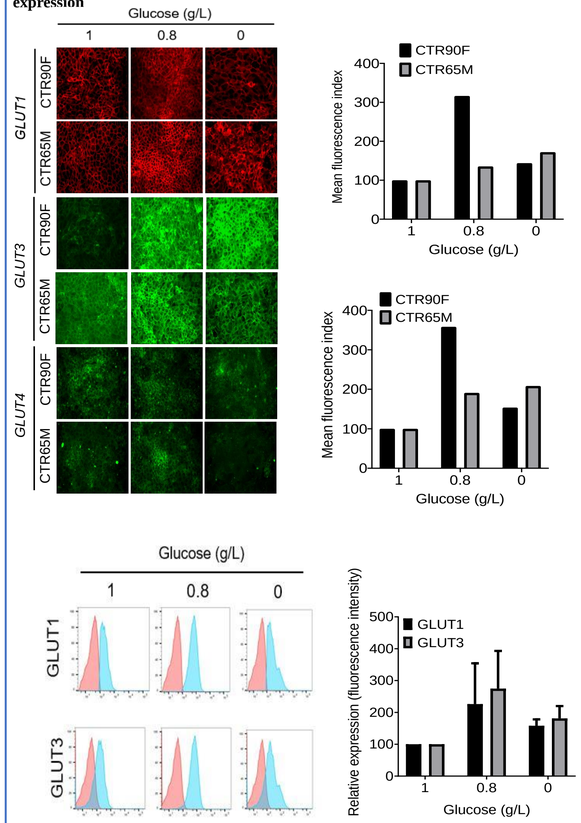


Figure 3: iPSC derived BMEC monolayers show differences in bioenergetics profile at different concentrations of glucose

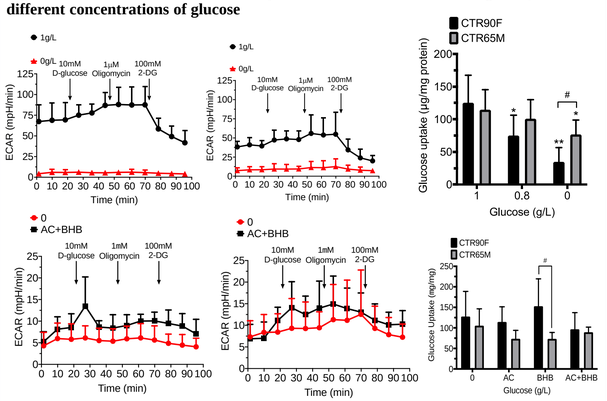


Figure 4: Quantitative analysis of beta hydroxybutyrate in *CTR-90F BMEC monolayers*

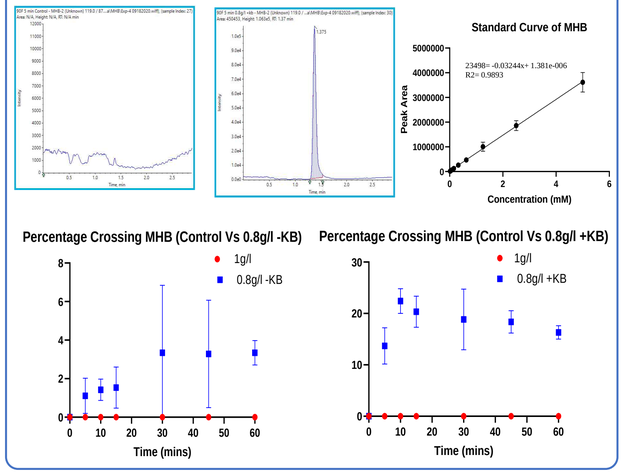


Figure 6: *iPSC derived BMEC monolayers show differences in BBB phenotype at different concentrations of glucose*

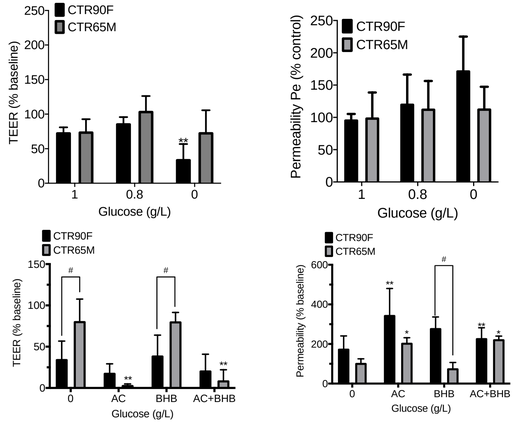
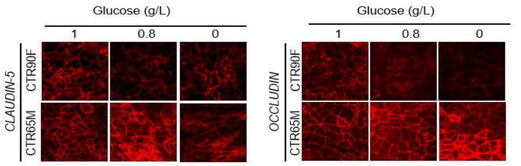


Figure 5: *iPSC derived BMEC monolayers show differences in tight junction profile at different concentrations of glucose*



CONCLUSION

- Decrease in glucose level upregulates the expression of GLUT1 and GLUT3 isoforms in our BMECs monolayers
- Decrease in glucose level is accompanied by decrease in glucose uptake, alterations in tight junction complex, as well as a decreased cell metabolic activity and glycolytic flux Under mild hypoglycemia, there is partial recovery of the barrier function and glycolytic flux
- BMECs may rely on glycolysis as the main source of energy, decrease in glucose results in poor barrier function which is partially relieved by ketone body supplementation

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