Modulation of Glucose Uptake in Corticotrophs via Transcriptional regulation of GLUT1

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Learning Objectives

By the conclusion of this session, participants should be able to: 1)

Describe the importance of ACTH production in glucose uptake by corticotroph cells. , 2) Discuss, in small groups, the mechanism of glucose regulation of corticotrophs.

Introduction

Preoperative detection of ACTH secreting tumors on imaging increases the chance of postoperative cure. FDG PET may detect some MRI negative tumors but not others. We studied the mechanism and modulation of glucose uptake in corticotrophs to understand the factors affecting glucose uptake.

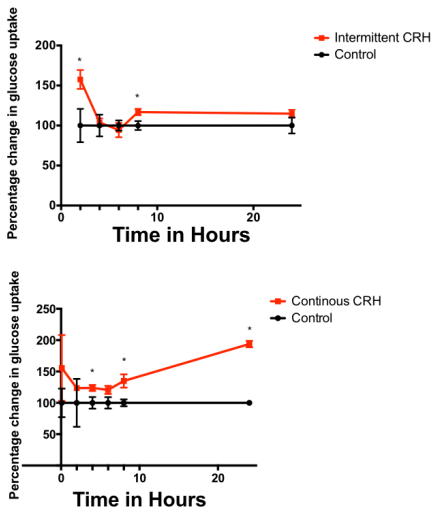
Methods

Murine corticotroph cells (AtT-20/D16-16) were used. ACTH was measured by ELISA. CRH (50nM) stimulated and unstimulated cells were incubated with deoxy-D-glucose for 1, 2, 4, 6, 8, and 24 hours to evaluate glucose uptake. Cells were stimulated with CRH continuously or briefly to reflect physiologic CRH effects. Quantitative real-time PCR (qRT-PCR) was used to investigate fold change in GLUT1, GLUT2, GLUT3, GLUT4, and POMC expression. Relative gene expression was calculated using the double -delta Ct method with ACTB expression as an internal control. POMC siRNA was used to perform knockdown studies.

Results

AtT20 cells have high basal uptake of glucose similar to glioma cell lines (U87, U251). CRH stimulation caused an increase in ACTH release by 290% ($p<0.05$). Continuous stimulation with CRH resulted in increased glucose uptake by 23%, 20%, 34%, and 94% at , 4,6,8, and 24 hours respectively ($p<0.05$). Timed stimulation with CRH for one hour to reflect clinical half-life of CRH resulted in increased glucose uptake by 57% and 16% at 2 and 8 hours respectively ($p<0.05$). CRH stimulation specifically up-regulated GLUT1 mRNA by 68% at 2 hours ($p<0.05$). GLUT1 was also upregulated by 94% at 2 hours following 1 hour timed stimulation by CRH. No consistent changes in expression of other glucose transporters were seen.

Figure 1

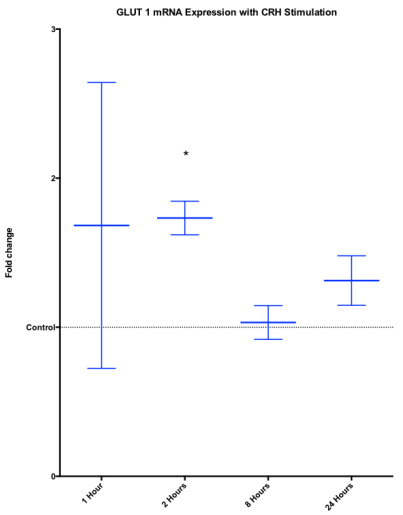


Timed (1 hour) and continuous stimulation of ATT20 cells with CRH resulted in an increase in uptake of labeled 2 Deoxy Glucose.

Conclusions

GLUT1 is responsible for glucose uptake regulation in corticotrophs. There appears to be a direct transcriptional regulation of GLUT1 by POMC. Physiologically timed CRH exposure may result in potent changes in GLUT1 expression.

Figure 2



qrtPCR revealed that GLUT1 expression is increased to nearly 2 fold in ATT20 cells with CRH stimulation. Such an increase was not seen in other GLUT (2, 3, 4) molecules.