Insulin promotes GCK activation and translocation through AKT-dependent mechanism

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Introduction

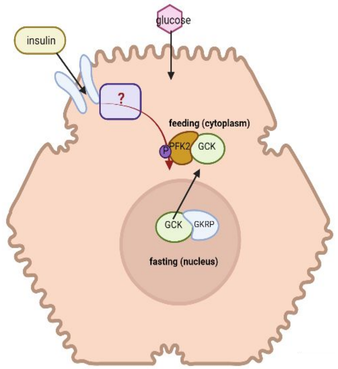


Figure 1: GCK is sequestered in the nucleus during fasting by glucokinase regulatory protein (GKRP) but translocates to the cytoplasm after a meal, where it binds with phosphofructokinase 2 (PFK2) and becomes catalytically active. Preliminary data shows that insulin increases phosphorylation of PFK2 at Ser486 in an AKT-dependent manner.This process of GCK translocation and subsequent activation is increased by insulin, although the mechanism through which this occurs is unknown.

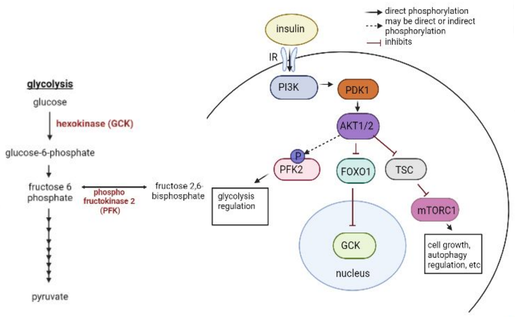


Figure 2: Simplified hepatic insulin signaling pathway through PI3K-AKT. Phosphorylation of PFK2 by AKT may occur directly or indirectly. Although it is known that insulin increases GCK expression, the method through which insulin (and AKT) acutely regulate GCK is unclear.

Question: What is the mechanism by which insulin promotes acute GCK translocation and activation

Hypothesis: Insulin promotes GCK translocation in hepatocytes by promoting GCK-PFK2 interaction in an AKT-dependent manner

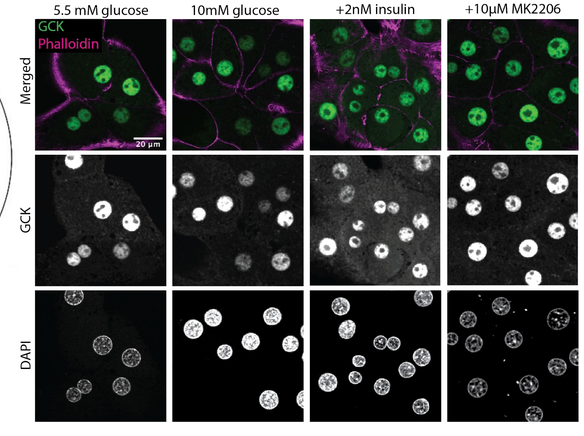
Methods

Three main methods were used to explore the impact of insulin on GCK activation and transport:

1. Immunofluorescence experiment was conducted to see if insulin has a significant impact on GCK translocation when high glucose levels are reflective of physiological conditions
2. Western blot was used to determine if insulin promotes PFK2 phosphorylation in an AKT-dependent manner in muscle as well as liver tissue
3. Nested PCR technique was used to clone GCK, GKRP, and PFK2 from rat cDNA

Results

Cytoplasmic translocation of GCK is promoted by insulin through AKT-dependent mechanism



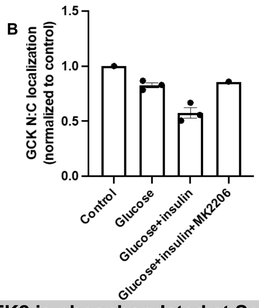


Figure 3:
A)Immunofluorescence of GCK in primary rat hepatocytes. hepatocytes were incubated overnight in low serum low glucose media without insulin, then pre-treated with AKT inhibitor (MK2206) or DMSO, and treated with high glucose (10mM) and 2nM insulin media +/- 10uM MK2206. Cells were incubated for 30 minutes, then washed, fixed, and stained. Green=GCK, magenta=phalloidin to indicate cell boundaries, DAPI=nuclei
B) Quantification of nuclear GCK to cytoplasmic (N:C), ratio normalized to control.

PFK2 is phosphorylated at Ser486 in hepatocytes in response to insulin in an AKT-dependent manner, but not in muscle

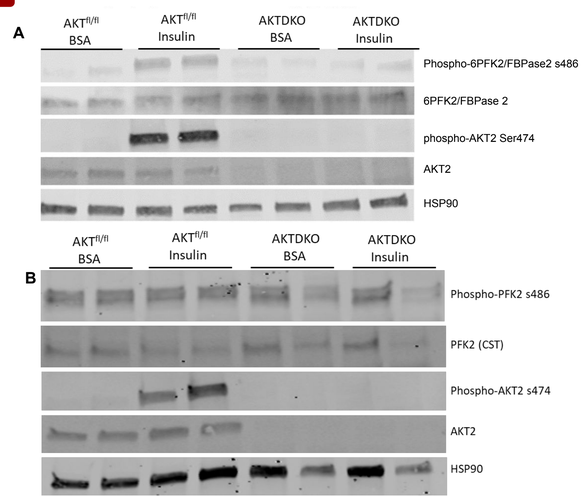


Figure 4:
A) In rat hepatocytes, phosphorylation of PFK2 at site Ser486 in response to insulin. Tested in rats lacking both AKT isoforms in the liver (AKTDKO) versus floxed controls (AKT fl/fl). Rats were fasted overnight, then challenged with insulin (0.75U/kg) after an overnight fasted and SAC'd 20 min later
B) In mouse AT skeletal muscle, phosphorylation of PFK2 at site Ser486 in response to insulin is not observed. Tested in mice lacking both AKT isoforms in the liver (AKTDKO) versus floxed controls (AKT fl/fl). Mice were fasted overnight, then challenged with insulin (0.75U/kg) after an overnight fasted and SAC'd 20 min later

Results

GCK and GKRP cloned from rat CDNA using nested PCR

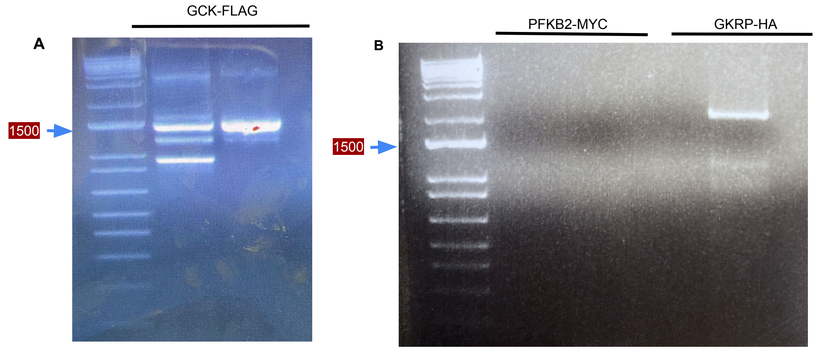
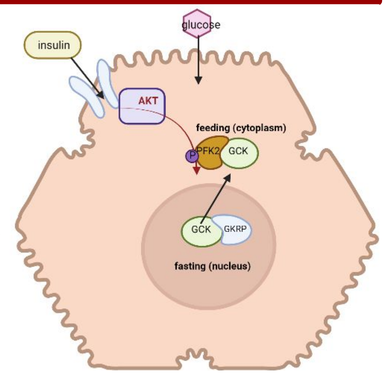


Figure 5:
A) Nested PCR of GCK with FLAG tag, insert is 1497bp
B) Nested PCR PFKB2 with MYC tag (not observable), and GKRP with HA tag (observable). insert is 1884bp

Conclusions and Future Directions

Conclusions:

1. Phosphorylation of PFK2 in response to insulin appeared in the liver sample but did not appear in the muscle sample, suggesting specificity to hepatocytes
2. The translocation of GCK was shown to be promoted by insulin through an AKT dependent mechanism at a glucose concentration of 10mM, suggesting that the mechanism has physiological relevance
3. GCK and GKRP were successfully cloned and tagged from rat CDNA



Future goals for rat CDNA cloning

- Overxpress tagged GCK and GKRP in an immortalized cell line to look at how PFK2 affects translocation by creating a mutated phosphorylation site (Ser486)
- Use tagged proteins in co-immunoprecipitation to enhance signal of naturally occurring GCK, GKRP, and PFK2 in hepatocytes to look at how PFK2 phosphorylation affects GCK-PFK2 interaction

Citations

1. *Chu et al. 2003. Am. J. Physiol.* 2. *Nozaki et al. 2020. PNAS* 3.*Payne et al. 2005. Diabetes*

Acknowledgements

Thank you to Megan Stefkovich and Dr. Paul Titchenell for their mentorship along with the other members of the Titchentell lab. Thank you to the CURF office for providing me the opportunity to be PURM scholar this summer