Determining the mechanism of GluT1 activation using caffeine inhibition

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Introduction

GluT1, a glucose transporter protein responsible for basal uptake of glucose in cells, is found in tissues throughout the body. Importantly, GluT1 expression is upregulated in many cancer cells, and the protein is also involved in Alzheimer's disease (1) and diabetic retinopathy (2). Understanding the properties of GluT1, and its regulation in particular, may therefore lead to new therapies for these diseases in the future.

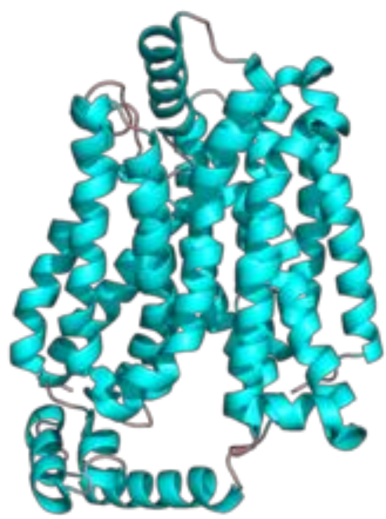


Figure 1. Cartoon depiction of the structure of GluT1 monomer.
https://en.wikipedia.org/wiki/GLUT1

GluT1 is most active as a homotetramer and a nucleotide-binding site is present only in this conformation (3). Caffeine binds to the nucleotide-binding site and is a dose-dependent uncompetitive inhibitor of GluT1 in erythrocytes (3). The effects of caffeine in cells with lower concentrations of GluT1, however, was unknown.

Curcumin is another inhibitor of glucose uptake through GluT1 and was hypothesized to bind at or near the nucleotide-binding site (4). Conversely, depriving cells of glucose acutely activates glucose uptake through GluT1 (5). One proposed mechanism of activation was GluT1 tetramer formation (5).

References

(1) Winkler et al. (2015). GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nature Neuroscience*. 18:521–530.

(2) Lu et al. (2013). Suppression of GluT1; a new strategy to prevent diabetic complications. *J Cell Physiol*. 228 (2):251-257.

(3) Sage et al. (2015) Caffeine inhibits glucose transport by binding at the GLUT1 nucleotide-binding site. *Am J Physiol Cell Physiol*. 308(10):C827-C834.

(4) Gunnink et al. (2016). Curcumin directly inhibits the transport activity of GLUT1. *Biochimie*. 125:179-185.

(5) Roelofs et al. (2006). Acute activation of glucose uptake by glucose deprivation in L929 fibroblast cells. *Biochimie*. 88:1941-1946.

Objectives

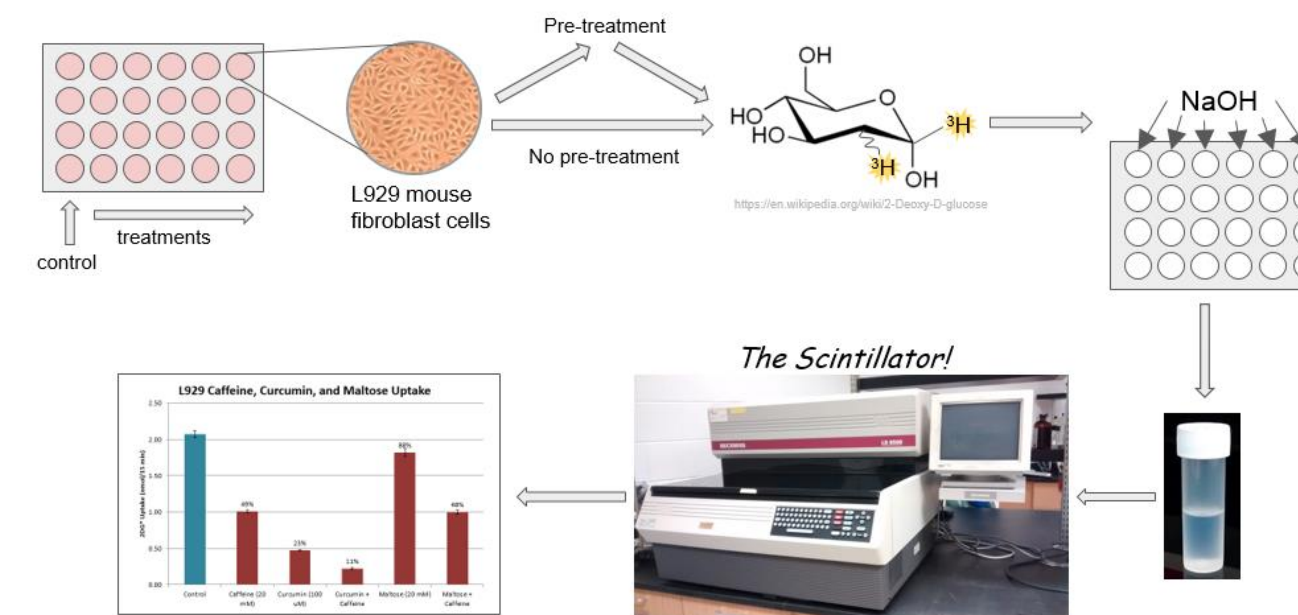
- Measure the effects of caffeine on GluT1 activity in L929 fibroblast cells and determine if the magnitude of the effect changes upon activation
- Measure the combined effects of caffeine with other inhibitors to begin to elucidate inhibitor binding sites

Methods

L929 mouse fibroblast cells

- Express GluT1 exclusively
- Lower concentrations of GluT1 than erythrocytes
- Compounds can be used to inhibit and activate GluT1 activity in L929s

Glucose Uptake Assay



- Cultured L929 cells in media with or without treatments
- Replaced media with solution of radiolabeled 3H-2-deoxyglucose (3H-2DG) and incubated for 15 minutes
- Washed off radioactivity and lysed cells
- Cell lysate collected and mixed with scintillation fluid
- Level of radioactivity in each lysate solution measured by scintillation spectrometry

Results

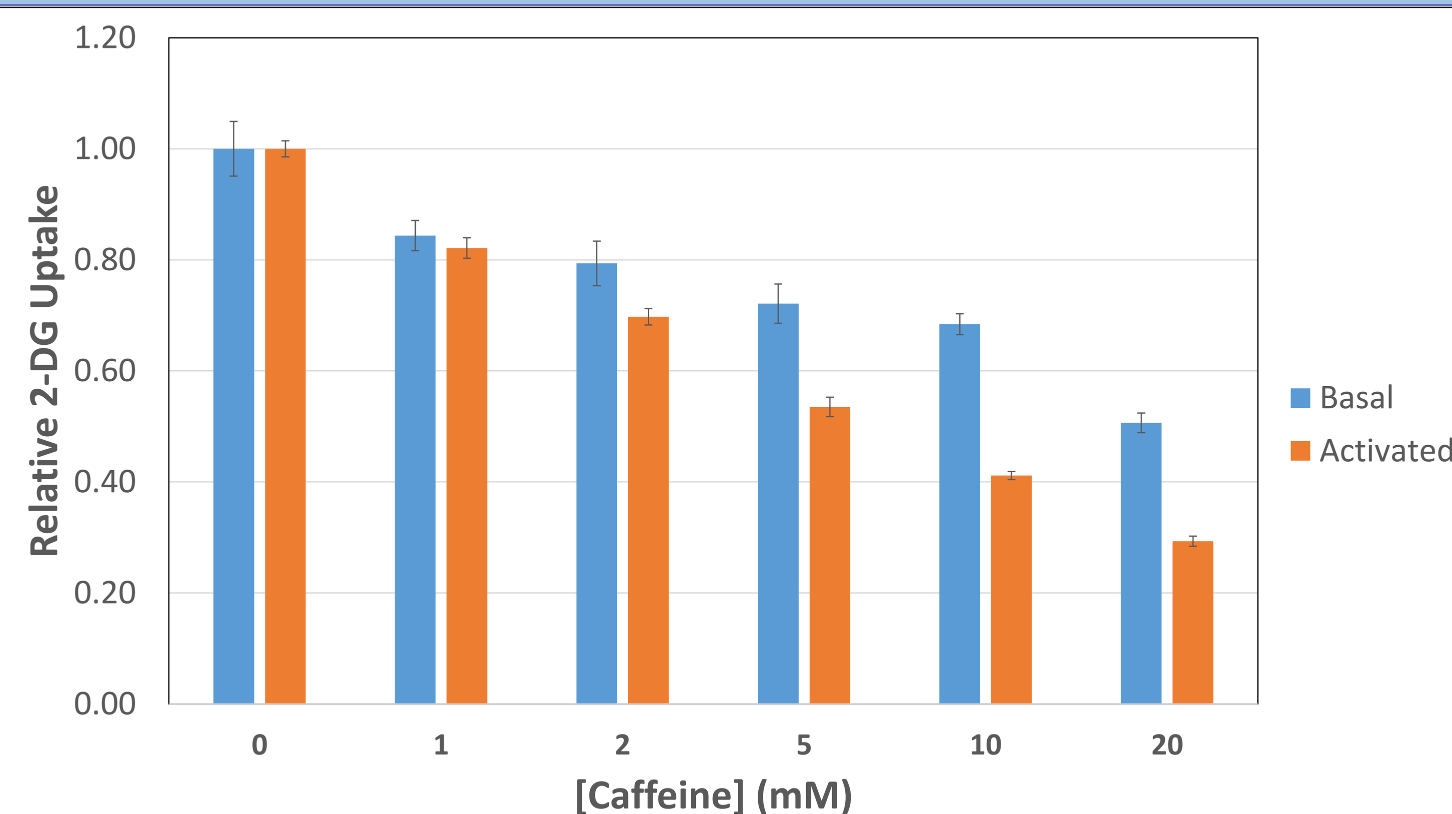


Figure 2. Caffeine concentration vs. relative 3H-2DG uptake in L929 cells under basal conditions and activated with no-glucose media. Caffeine inhibition of 3H-2DG uptake is more robust when L929 cells were treated with no glucose media.

Results cont.

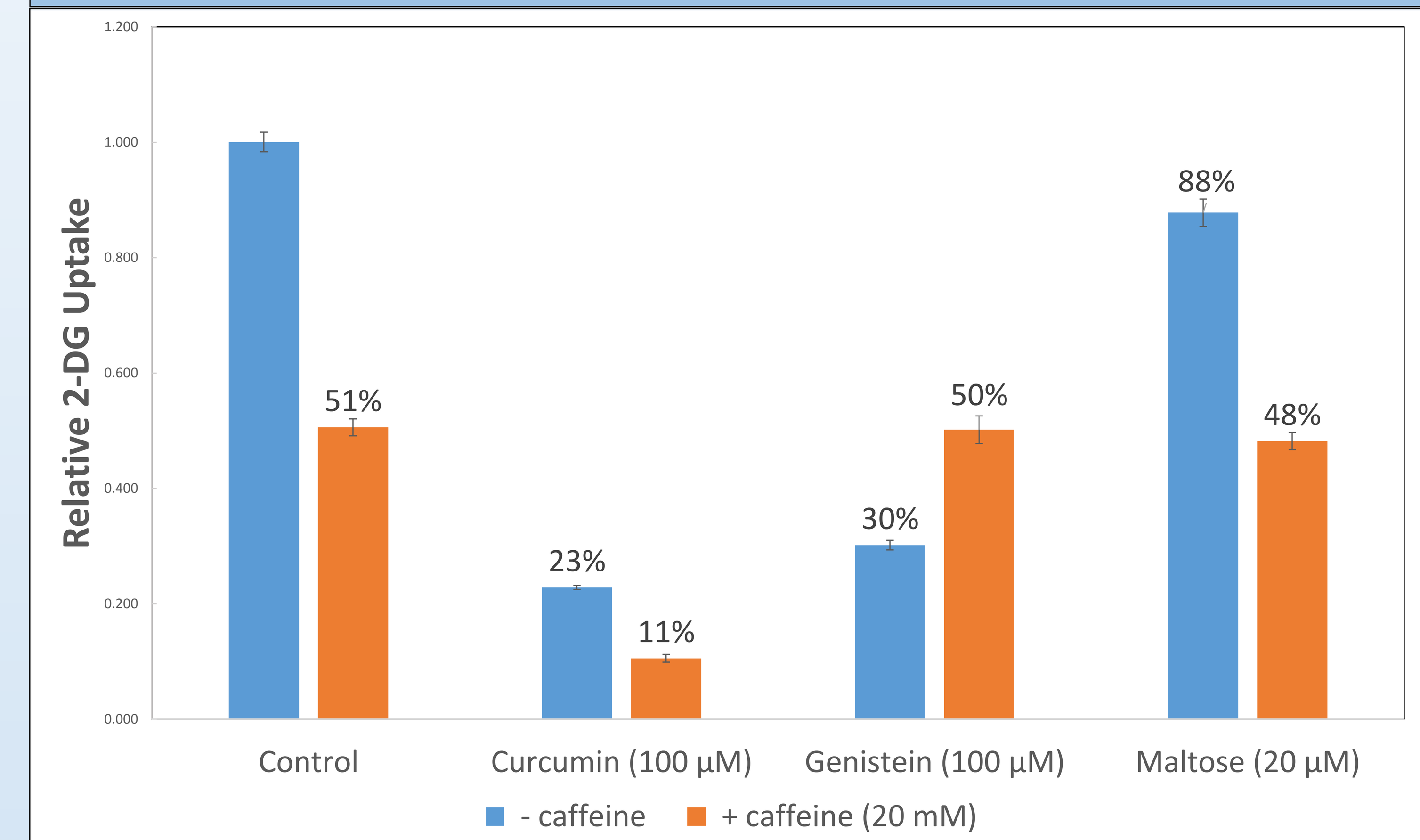


Figure 3. Relative 2-DG uptake for L929 cells treated with 100 µM curcumin, 100 µM genistein, and 20 µM maltose with and without 20 mM caffeine. Uptake percentages are the average of two uptake assays with four replicates per treatment per assay. The raw data was normalized to the control for each assay. Curcumin inhibition was additive to the caffeine inhibition. Genistein inhibition was rescued by the presence of caffeine. Maltose inhibition was inconclusive.

Conclusions

- Glucose deprivation induces GluT1 tetramer formation, as evidenced by the increased inhibition by caffeine in L929 cells treated with NG media. Caffeine binds only to tetrameric GluT1, so an increase in relative inhibition indicates a greater proportion of GluT1 tetramers.
- Caffeine inhibition is additive to curcumin inhibition of GluT1, therefore, curcumin does not bind to the GluT1 nucleotide-binding site in the manner that caffeine does.
- Genistein inhibition is decreased by the presence of caffeine. It may be that the caffeine binding changes the conformation of GluT1 in such a way that the genistein cannot bind as well, and thus inhibition is decreased.

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