The Effect of pH on GluT1 Activity
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Introduction

GluT1 is a trans-membrane protein which transports glucose into cells though facilitative diffusion. The mechanism for activation of this transport is unknown.

GluT1 is ubiquitous in mammalian cells and therefore has implications in cancer and diabetes. The ability to deactivate GluT1 in cancer cells and activate GluT1 in diabetic cells has potential applications in treatment plans.

Methods

• L929 mouse fibroblast cells were plated on 24- or 48-well plates for 1-2 days
• In some experiments, cells were treated with activating conditions for 30 minutes
• Glucose uptake was measured by exposing cells to the radioactive glucose analog, 2-deoxy-D-glucose (1,2-3H) (2DG) for 10 minutes at various pHs as indicated.
• Cells were then washed and lysed with .5M NaOH and the amount of radioactive 2DG was measured using scintillation spectroscopy.

Results

Figure 1. The effect of pH on glucose uptake. A. Glucose uptake was performed at different pH between pH 4 - pH 10. A 5 to 6 fold activation was observed from pH 6 to pH 8 with no significant change outside that range. B. Cells were pretreated with 1.0 mM iodoacetamide (IA), a compound that reacts with thiols. No pH effect was observed. Data suggest cysteine residues with pKa>7.5 are required for activation.

Figure 2. The combined effect of pH with thiol reactive activators. Phenylarsine Oxide (PAO) and Hydroxylamine (HA) were added to the radioactive glucose at varying pH. PAO and HA activate GluT1 at pH 6-7 but have no further activation at pH 8.

Figure 3. The combined effect of pH with stress-activators of glucose uptake. Glucose uptake was performed on cells previously treated with berberine, glucose deprivation, and methylene blue. An additive effect was observed for berberine and methylene blue, but for glucose deprivation.

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Conclusion

• Alkaline pH activates GluT1.
• The pH effect is blocked by prior treatment with IA to block free thiols.
• Thiol reactive activators are unable to activate at pH 8.
• pH effects are additive to the long term activators, berberine or methylene blue, but not to the more robust activating effects of glucose deprivation.
• Activation from pH 7 to 8 does not occur in cells where GluT1 is already highly active.
• Data suggest that pH effects involves the thiol group of the cysteines and are consistent with the working model.

References