Exploring the Inhibition of GluT1 by Caffeine

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The glucose transporter isoform 1 (GluT1) protein is a transmembrane protein that transports glucose into and out of cells. It is found in many different kinds of cells all throughout the human body, and problems with its regulation are connected to several major diseases, including Alzheimer's, diabetes, and cancer. Cancer cells in particular overexpress GluT1 to meet their increased need for glucose as they rapidly grow and divide. Understanding how GluT1 is regulated may therefore be useful if GluT1 becomes a target for cancer therapies.

Compounds like curcumin and caffeine inhibit GluT1 and slow glucose uptake into cells. Treating cells with berberine or no glucose media, however, will activate GluT1 and increase glucose uptake. The Louters lab has previously studied regulation of GluT1 by all of these treatments except caffeine. GluT1 can aggregate into dimers (two proteins bound together) and tetramers (four proteins bound together), and it is in the tetramer form that GluT1 is most active. It is also in tetrameric GluT1 that an ATP-binding site becomes available, and this is where caffeine binds.

Red blood cells, which other research teams have used to study GluT1, contain a very high concentration of GluT1 in their cellular membrane, and it is hypothesized that most of the GluT1 in these cells is in the tetrameric form and able to bind caffeine. However, we were interested in measuring GluT1 regulation, particularly by caffeine, in cells that had lower membrane concentrations of GluT1. Therefore, we used L929 mouse fibroblast cells in our experiments. These cells express only GluT1 and have a much lower concentration of the protein than red blood cells do. (Many other body cells express a cocktail of the other 13 isoforms that is specific to their function.)

Because we know the site where caffeine binds to GluT1, we can also begin to determine the binding sites of other inhibitory compounds. Two inhibitors that bind to the same site will compete with each other for that site. Adding the second inhibitor to the first will not change the amount of inhibition measured. Two inhibitors that bind to different sites, however, will bind simultaneously, and adding a second inhibitor to the first will increase inhibition. In this case, the inhibitory effects are additive.

To measure the activity of GluT1 under different conditions, we carried out glucose uptake assays with L929 mouse fibroblast cells. We grew the cells in 24-well plates until the cells covered the surface of the wells. The cells were then treated with no glucose media to induce GluT1 activation, or they were used in the assay without pre-treatment. In the uptake portion of the assay, the cells were incubated in a solution of radiolabeled [1,2-^{3}H]-2-deoxyglucose (2DG) for 15 minutes. Then the radioactivity was washed off, the cells were lysed, and the amount of radioactive glucose taken up by the cells was measured using a liquid scintillation counter. The amount of radioactivity counted is directly proportional to the amount of radioactive glucose taken up in the 15-minute incubation period.

Using this uptake assay, we determined that caffeine inhibition is more robust when cells are activated with no glucose media, implying that this pre-treatment increases the concentration of tetrameric GluT1 in the cellular membrane. We also found that inhibition by curcumin is additive to inhibition by caffeine, indicating that curcumin does not bind to the same site on GluT1 as caffeine. This was an unexpected discovery, as there are many similarities in the inhibitory patterns of curcumin and caffeine, and it had been suggested

that curcumin might also bind to the ATP-binding site. Finally, we determined that caffeine does not compete with 2DG transport through GluT1. The inhibition caused by glucose (the molecule that GluT1 naturally transports, and a competitive inhibitor of radiolabeled 2-deoxyglucose) is additive to inhibition by caffeine.

Participating in the ongoing GluT1 research under Professors Louters and Looyenga gave me experience in the scientific process of asking questions, experimenting, reporting results, and asking new questions. Several of the experiments that we tried failed initially or produced unexpected results. Learning to move past failures and interpret unforeseen results has strengthened my resilience and broadened my perspective. My research experience has also reinforced my desire to pursue graduate studies in the biochemistry field. Above all, I have been amazed by the ordered complexity with which God imbued creation and by the relatively simple tools we can use to manipulate and understand the workings of some of the smaller pieces of this creation.