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A Study on Biological Functions of Glut 4

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Abstract – Glucose Transporter 4 (GLUT4) is a twelve transmembrane protein of the Major Facilitator Super family that facilitates the diffusion of D-glucose across the cell membrane. GLUT4 is predominantly expressed in adipocytes and muscle cells, which are the major sites of postprandial glucose disposal in the body. Among the different glucose transporter isoforms, GLUT4 is distinguished by its intracellular distribution and responsiveness to the hormone, insulin. The protein synthesized is targeted to distinct intracellular compartments and is acutely redistributed to the plasma membrane in response to various stimuli. Upon stimulation with insulin, an intricate signaling cascade occurs in these cells which results in the movement of these vesicles towards the plasma membrane.

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INTRODUCTION

Glucose transporter type 4, also known as **GLUT4**, is a protein that in humans is encoded by the *GLUT4* gene. GLUT4 is the insulin –regulated glucose transporter found in adipose tissues and striated muscle (skeletal and cardiac) that is responsible for insulin-regulated glucose transport into the cell. This protein is expressed primarily in muscle and fat cells, the major tissues in the body that respond to insulin. The first evidence for this distinct glucose transport protein was provided by David James in 1988.The gene that encodes GLUT4 was cloned and mapped in 1989.

The ability to transport glucose across the plasma membrane is a feature common to nearly all cells, from simple bacteria through to highly specialised mammalian neurones. Facilitative sugar transport is mediated by members of the GLUT transporter family, which form an aqueous pore across the membrane through which sugars can move in a passive (i.e., energy-independent) manner; in consequence, they can only transport sugars down their concentration gradient. The GLUT family of glycosylated transmembrane proteins are predicted to span the membrane 12 times with both amino- and carboxyl-termini located in the cytosol. On the basis of sequence homology and structural similarity, three subclasses of sugar transporters have been defined: Class I (GLUTs 1-4) are glucose transporters; Class II (GLUTs 5, 7, 9 and 11) are fructose transporters; and Class III (GLUTs 6, 8, 10, 12 and HMIT1) are structurally atypical members of the GLUT family, which are poorly defined at present, indeed GLUT6 may only be a pseudo-gene.

The confirmed isoforms are expressed in a tissue and cell-specific manner, and exhibit distinct kinetic and regulatory properties, presumably reflecting their specific functional roles. They belong to a much larger 'major facilitator superfamily' of 12 TM transporters that are involved in the transport of a variety of hexoses and other carbon compounds, and include: bacterial sugar-proton symporters (H^+/xylose and H^+/arabinose); bacterial transporters of carboxylic acids and antibiotics; and sugar transporters in various yeast, protozoa and higher plants. Nevertheless, amino acid identity within the super family may be as low as ~25% Besides the 12 presumed TM domains; the most characteristic structural feature of the super family is a five residue motif (RXGRR, where X is any amino acid). In the GLUT transporters, this motif is present in the presumed cytoplasmic loops connecting TM domains 2 with 3, and also 8 with 9. The 12 TM transporter superfamily appears to be structurally unrelated to the Na^+-coupled, Na^+/glucose co-transporters (SGLT1-3) found in the intestine and kidney, which are able to transport glucose against its concentration gradient.

Comparison of the hydropathy profiles for GLUT1-5 reveals that they are virtually super imposable, despite the fact that their primary structures may differ by up to 60%. Of the presumed TM domains, the fourth, fifth and sixth are the most highly conserved, and conserved residues are also found in the short exofacial loops joining the putative TM regions. The presumed cytoplasmic N- and C-termini, and the extracellular loop between the first and second TM domains, show the greatest divergence, both in terms of primary structure and size.

GLUT4 is thought to be an insulin-responsive glucose transporter, expressed in the membranes of the cells and organelles of skeletal muscle, heart and fat. These tissues are insulin-sensitive and respond to increased blood insulin levels by a rapid and reversible 20-30 fold increase in glucose transport.

This is thought to be brought about (at least partially) by the translocation of a latent pool of glucose transporters from an intracellular site to the plasma membrane. On entry into the endosomal system, GLUT4 is selectively retained at the expense of other recycling transport that constitutively moves between the endosomes and the cell surface. This retention mechanism might predispose GLUT4 for sorting into transport vesicles that bud slowly from the endosome and that are targeted to the trans-Golgi network (TGN). GLUT4 is sorted into a secretory pathway in the TGN. This probably involves a specialised population of secretory vesicles that excludes other secretory cargo, and that does not fuse constitutively with the plasma membrane. In the absence of insulin, GLUT4 storage vesicles might slowly fuse with endosomes, thereby accounting for the presence of a significant but small pool of GLUT4 in endosomes, even in the absence of insulin. Insulin would then shift GLUT4 from this TGN-endosome cycle to a pathway that takes GLUT4 directly to the cell surface.

RESEARCH METHODOLOGY:

GLUT4 consists of 509 amino acids (human isoform) and shows ~60% amino acid identity to the GLUT1-3 isoforms, being most similar to GLUT1. Both the N- and C-terminal portions of the molecule having been reported to be involved in the targeting.

The abundance of GLUT4 at the plasma membrane in turn facilitates the bulk transport of sugar in to these cells. A second mechanism known as GLUT4 activation also takes place in response to insulin which results in an increased catalytic activity of the transporters that are at the cell surface. Therefore by mediating insulin responsive glucose transport, this polytopic membrane protein plays a phenomenal role in the regulation of whole body glucose homeostasis. Any molecule that increases the GLUT4 exocytosis and/or intrinsic activity facilitates the increased glucose entry into the cells and such molecule would have therapeutic potential to treat type 2 diabetes, a metabolic disorder characterized by enhanced blood glucose levels. The present work is an attempt to identify novel modulators of GLUT4 translocation and GLUT4 mediated glucose transport from natural products and delineate their molecular mechanism of action.

In order to find novel modulators of glucose transport from natural products, a group of 50 medicinal plants were selected for the initial screening studies after a detailed literature survey. Petroleum ether and methanol extracts of the plant parts were prepared, systematically labeled and stored. The extracts were screened for their effect on radio-labelled glucose uptake in 3T3-L1 adipocytes. Some of the extracts displayed significant modulatory effects on glucose transport. Among these, the methanol extract prepared from the leaves of Terminalia arjuna exhibited a considerable increase in glucose uptake activity similar to insulin. Terminalia arjuna is a deciduous tree widely distributed throughout India and a well known ayurvedic medicine for the treatment of various illnesses. A systematic fractionation of the extract was carried out to isolate the active principle/s. For that, the methanol extract was subjected to activated charcoal treatment and acid hydrolysis followed by silica column chromatography. Bioassay directed fractionation resulted in the identification of ellagic acid as one of the active compounds. The second active compound isolated was gallic acid. Since gallic acid has already been reported to possess stimulatory effect on GLUT4 translocation and glucose transport in 3T3-L1 adipocytes, our subsequent studies were focused on ellagic acid.

REVIEW OF LITERATURE:

Ellagic acid is a polyphenol naturally occurring in berries and nuts mainly as ellagitannins. Ellagic acid was found to stimulate glucose transport in both 3T3-L1 adipocytes and C2C12 myotubes in a dose dependent manner. GLUT4 translocation assay in 3T3-L1 fibroblasts stably transfected with a myc-GLUT4-GFP construct demonstrated a concomitant increase in GLUT4 translocation upon ellagic acid treatment. The effect of ellagic acid on the phosphorylation status of the major proteins involved in the insulin signaling cascade was analyzed. Neither Protein Kinase B/Akt nor Akt Substrate of 160 kDa (AS160) phosphorylation was enhanced in the presence of ellagic acid. However protein phosphorylation studies suggested the activation of AMP activated protein kinase (AMPK) in response to ellagic acid treatment in both cell lines. AMPK pathway has profound importance in mediating GLUT4 translocation in an insulin independent manner. Analyzing the downstream signaling events suggested an activation of extracellular signal regulated kinase (ERK1/2) and atypical PKC ζ/λ (aPKC ζ/λ). Inhibitor studies have shown inhibition of ellagic acid stimulated glucose transport in the presence of AMPK inhibitor, compound C and ERK inhibitor, PD98059. Application of compound C also blocked EA induced ERK phosphorylation. These results suggest that ellagic acid mediates its metabolic effect on glucose transport through a mechanism distinct from that of insulin and involves AMPK pathway.

Arjunolic acid is a triterpenoid compound abundantly present in the heavy wood of Terminally Arjuna. Triterpenoids form an important class of plant secondary metabolites and they possess a wide range of biological activities. Some triterpenoids are known to exert significant modulatory effect on glucose transport. In the present work we analyzed whether arjunolic acid has any effect on glucose transport in 3T3-L1 adipocytes. It was found that arjunolic acid inhibits insulin stimulated glucose uptake in 3T3-L1 adipocytes when prior treated with insulin for thirty minutes. The inhibition was not due to a reduction in GLUT4 translocation as observed from the GLUT4 translocation assay. This was further

confirmed by analyzing the phosphorylation status of key kinases involved in the insulin signaling pathway, Akt and aPKCζ/λ. Next attempt was to analyze whether arjunolic acid affected insulin mediated known intrinsic activation pathways. P38 mitogen-activated protein kinase (p38MAPK) and p44/p42 mitogen-activated protein kinase (p44/42 MAPK) are the two reported pathways proposed to play a role in the insulin stimulated intrinsic activation of GLUT4. So we examined the phosphorylation status of p38MAPK and p44/42 after insulin treatment in the presence and absence of arjunolic acid. Neither insulin simulated p38MAPK nor p42/44MAPK activation was reduced by the presence of arjunolic acid. These results suggest that the inhibitory action of arjunolic acid on insulin stimulated glucose transport is not mediated through affecting any of the canonical insulin signaling events. Therefore we speculated that arjunolic acid could be inhibiting glucose transport through a direct interaction with the transporter. To check this possibility, we added the compound to insulin stimulated cells only at the time of the glucose transport assay. Here also we could observe a substantial inhibition of insulin stimulated glucose transport suggesting a direct interaction of arjunolic acid with GLUT4.

SIGNIFICANCE OF THE STUDY:

Using a well validated homology model of GLUT4, our laboratory had carried out an in silico screening study for identifying potential ligands of GLUT4. From this study, a compound (henceforth referred to as Compound S) was already shown to possess stimulatory effect on glucose transport in 3T3-L1 adipocytes. A further study was carried out to characterize the action of this compound. The compound was found to possess a dual role in this stimulatory action. At one level it found to stimulate the translocation of GLUT4 to the cell surface. The increase in GLUT4 translocation was mediated through activation of atypical PKCζ/λ in a Phosphoinositide-3-Kinase (PI3K) dependent manner. At a second level the compound was also found to directly stimulate the intrinsic transporter activity of GLUT4. This raised another question; although arjunolic acid and compound S bind at the same site of the transporter, why do they show antagonistic effects on glucose transport? To gain further insight into the possible mechanism of action of compound S, we looked at the dynamic behavior of the transporter in the presence of compound S. The pore radius analysis of the transporter during the 20 ns simulation with compound S in the presence of D-glucose has shown a tendency of the transport channel to open at the cytoplasmic face. There was absence of an inter-domain salt bridge switching which was observed in the case of arjunolic acid and ATP. The inter-helical hydrogen bonding pattern is also different with respect to ATP and arjunolic acid. The transport channel analysis, salt bridge and inter-helical hydrogen bonding propose a conformation where the channel has a tendency to open towards the cytosolic face which may be responsible for the stimulatory effect of this compound.

CONCLUSION:

In conclusion, this study has led to the identification of few molecules with significant modulatory effects on glucose transport. Two of the compounds namely, ellagic acid and compounds were found to activate glucose transport through two different mechanisms. Arjunolic acid exhibited inhibition of insulin stimulated glucose transport and further analysis proved that this inhibition was due to binding of the compound at the ATP binding site and thus plays a role in closing the glucose permeation channel. Further studies are needed to understand the effect of these compounds in vivo.

The docking studies of arjunolic acid with a well validated GLUT4 homology model generated in our lab have revealed the binding of the molecule at a putative ATP binding motif (GRRLTHL) present in the loop connecting TM helices 8-9. However there was no interaction at the QLS motif, which is the glucose binding site present in GLUT4. This finding prompted us to look further into the mechanistic details of this interaction. Molecular dynamics simulation studies have revealed an inter-domain salt bridge switching and inter-domain hydrogen bond formation similar to those observed during simulation studies performed with ATP. Switching of salt bridge, formation of inter-domain hydrogen bonds and pore radius analysis suggested constriction of the glucose transport channel at the cytoplasmic face of the transporter which may be responsible for this inhibitory effect. To ascertain this direct interaction, we performed a 8-azido ATP [γ] biotin labeling study. UV irradiation at 254 nm caused labeling of GLUT4 with 8-azido ATP[γ] biotin and this binding was reduced in the presence of ATP and arjunolic acid suggesting a competition with 8-azido ATP for the same binding site.

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