Glucose transporter 4 (glut4) and diabetes

Insulin and AMP-activated protein kinase (AMPK) signal pathways are known to be involved in glucose uptake regulation, but the integration of signals between these two pathways in maintaining glucose homeostasis remains mysterious (1). Insulin stimulates glucose uptake into skeletal muscle mainly via the translocation of glucose transporter 4 (GLUT4) to the plasma membrane (2). Also the exact mechanism involved is not yet fully understood, however, in insulin-induced glucose transport cascade it seems that insulin activates specific protein kinase C (PKC) isoforms (2).

In Liu L. Z. et al work, stimulation of insulin conferred a glucose uptake or translocation of surface glucose transporter 4 (GLUT4). Using specific inhibitors to key kinases of both pathways and PKCzeta small interference RNA, it was found that protein kinase C zeta (PKCzeta)regulate insulin-stimulated protein kinase B (PKB) activation and inhibit AMPK activity on dorsal cell surface, whereas inthe presence of berberine, PKCzeta controlled AMPK activation and AMPK blocked PKB activity in perinuclear region. The inhibitory effect exerted by PKCzeta on AMPK activation or the arrestment of PKB activity by AMPK still existed in basal condition. Overall, these results are suggestive for an antagonistic regulation between insulin and AMPK signal pathways, which is mediated by the switch roles of PKCzeta.

The protein kinase Akt is associated with different cellular processes, including cell proliferation, growth, metabolism and importantly, insulin-regulated transport of glucose into muscle and fat cells. Activation of Akt involves binding of an extracellular ligand to its cognate tyrosine kinase receptor, which lead to activation of PI3K (phosphoinositide 3-kinase) and generation of PtdIns(3, 4, 5)P3. This in turn enables the translocation of Akt

to the plasma membrane where it is phosphorylated at Thr308 and Ser473 by PDK1 (phosphoinositide-dependent kinase 1) and the mTOR (mammalian target of rapamycin)/rictor (rapamycin-insensitive companion of mTOR) complex respectively. Once activated, Akt phosphorylates a range of substrates that regulate a variety of key biological processes (3). There are several Akt isoforms, Akt1 function is mainly related to growth and proliferation. On the other hand, Akt2 is more involved in metabolism, and loss-of function mutations in Akt2 have been linked to Type 2 diabetes (3). Akt has been involved in the insulin-induced expression of GLUT1 in hepatoma cells and GLUT3 in skeletal muscle (4).

Insulin-regulated transport of glucose into muscle and fat cells is thought to be mediated by Akt-dependent movement of GLUT4 glucose transporters to the plasma membrane (3). It was found after Tan S. X. study that non-ATP-competitive allosteric Akt inhibitors in 3T3-L1 adipocytes caused a decrease in the Akt signaling pathway simultaneously with reduced glucose uptake, notwithstanding, no such reduction in GLUT4 translocation to the plasma membrane was seen. This is explained by the fact that minimal amount of Akt phosphorylation is prerequisite for robust GLUT4 translocation, thus a marked reduction in Akt phosphorylation may not necessarily translate into a similar reduction in GLUT4 translocation to the plasma membrane (3, 5). Additional investigation revealed that the inhibitory effects on glucose uptake in 3T3-L1 adipocytes of these compounds were independent of the Akt signaling pathway. Moreover, inhibition of glucose transport was also noticed in other cell types such as human erythrocytes that are rich in GLUT1 and T-47D breast cancer cells, proposing that these effects are not specific

to GLUT4, and that the Akt inhibitors affect glucose uptake in different cell types (3).

Alternatively, the phosphatidylinositol 3-kinase (PI3K) signal transduction pathway is a well-known mediator of cell growth, proliferation, and survival signals. Riley J. K. et al studied the Inhibition of the PI3K pathway, and results indicated that inhibition of this pathway cause an induction of apoptosis in both murine blastocysts and trophoblast stem cells. Moreover, the apoptosis induced correlates with a decrease in the expression of the glucose transporter GLUT1 at the plasma membrane (6). Additionally, blastocysts cultured in the presence of the PI3K inhibitor LY-294002 exhibited a reduction in 2-deoxyglucose uptake and hexokinase activity.

In rat primary brown adipocytes, insulin acutely stimulated glucose uptake in a PI3-kinase-dependent but p70S6-kinase-independent manner, and Akt represents an intermediate step between these kinases. Therefore, the goal of Hernandez R. et al study was to investigate the contribution of Akt to insulin-induced glucose uptake in brown adipocytes using ML-9, a recently proposed chemical inhibitor of Akt activity (7) and by using Akt protein with dominant-negative activity to block (4). ML-9 treatment of primary brown adipocytes showed a complete inhibition of insulin-stimulated glucose uptake and also impaired GLUT4 redistribution from internal membrane to plasma membrane in response to insulin. It was found that ML-9 inhibited Akt activity and Akt phosphorylation at Ser473. Furthermore, results showed that long-term treatment with ML-9 produced down-regulation of the GLUT4 mRNA accumulation, inactivation of Akt, and possibly its translocation to the nucleus (8). Co-transfection with ÏªAkt prevented insulin stimulation of GLUT4

promoter activity, suggesting that PI3-kinase/Akt pathway is regulating GLUT4 gene transcription by insulin (4).

It is well known that phosphatidylinositol (PI)3-kinase activation mediates GLUT4 redistribution to the plasma membrane, and overexpression of the catalytic subunit of p110 produced GLUT4 translocation and increased glucose uptake (9). Alternatively, inhibition of PI3-kinase (by chemical inhibitors or by microinjection of blocking p85 protein, or by transfection with a dominant-negative mutant of p85) prohibits insulin-induced GLUT4 translocation (10).

Two classes of Ser/Thr kinases have been suggested to act downstream of PI3-kinase, and they are Akt/PKB and the atypical protein kinase (PK) C isoforms ζ and λ (PKC ζ/λ). It well established that expression of a constitutively active, membrane-bound form of Akt cause a persistent localization of GLUT4 at the plasma membrane and increased glucose uptake in 3T3-L1 adipocytes, yet, it did not promote GLUT4 translocation or glucose transport in L6 myotube(4, 11).

Despite the fact proved by in vivo studies that GLUT4 expression in insulin-responsive tissues is under insulin and/or metabolic control (12), experiments performed with cultured fat cells had failed to establish a stimulatory role of insulin in GLUT4 expression (13), and the positive effect of insulin seen on GLUT4 gene expression had only been noticed in the presence of dexamethasone (14). Nevertheless, rat cardiomyocytes showed a direct effect of insulin on GLUT4 transcription (15).

Different studies have validated that insulin-stimulated glucose uptake is separated from GLUT4 translocation based on using phosphatidylinositol 3-kinase inhibitor; wortmannin . Therefore, it can be concluded that GLUT4 translocation and the intrinsic activity of GLUT4 are differentially regulated.

Insulin is known to activate p38MAPK, and using SB203580; p38MAPKα and p38MAPKβ inhibitors reduce insulin- stimulated glucose uptake without affecting GLUT4 translocation. p38MAPKs are serine/threonine kinases that are activated by several factors such as environmental stressors, inflammatory cytokines, protein synthesis inhibitors, growth factors like insulin and muscle contraction. It has four isoforms; p38α and p38β that are ubiquitously expressed, p38γ that is highly expressed in skeletal muscle and p38δ in the lung and kidney (16). Full activation of p38MAPK necessitates dual phosphorylation on residues corresponding to threonine-180 and tyrosine-182 on p38α catalyzed by dual-specificity MAPK kinases 3 and 6 (MKK3 and 6).

p38MAPK inhibitors like pyridinyl imidazole derivatives (SB203580 and SB202190) or azaazulene pharmacophores (A291077 and A304000) have the potential to inhibit insulin-mediated glucose uptake without producing any effect on GLUT4 translocation. As a conclusion, insulin increases the intrinsic activity of GLUT4 via p38MAPK (16).

Antonescu, C. N. et al aimed to determine whether p38MAPK is required for insulin- stimulated glucose uptake in L6-GLUT4myc myotubes using a mutant of p38α (DR-p38α) resistant to SB203580, dominant-inhibitory mutants of p38α and p38β and siRNA against p38α and p38β. The results obtained by

these independent molecular methods to interfere with p38MAPK signaling did not interfere with stimulated glucose uptake. Nonetheless, SB220025 was found to inhibit p38MAPK as strongly as SB203580, but again, had no effect on insulin stimulated glucose uptake. So, collectively, it can anticipated that p38MAPK is not involved in insulin-stimulated glucose uptake and that the SB203580-mediated reduction in glucose uptake is a result of SB203580 action on another protein (16).

References

1. Liu LZ, Cheung SC, Lan LL, Ho SK, Chan JC, Tong PC. The pivotal role of protein kinase C zeta (PKCzeta) in insulin- and AMP-activated protein kinase (AMPK)-mediated glucose uptake in muscle cells. Cellular signalling. 2010; 22(10): 1513-22. Epub 2010/06/24.

2. Braiman L, Alt A, Kuroki T, Ohba M, Bak A, Tennenbaum T, et al. Activation of protein kinase C zeta induces serine phosphorylation of VAMP2 in the GLUT4 compartment and increases glucose transport in skeletal muscle. Molecular and cellular biology. 2001; 21(22): 7852-61. Epub 2001/10/18.

3. Tan SX, Ng Y, James DE. Akt inhibitors reduce glucose uptake independently of their effects on Akt. The Biochemical journal. 2010; 432(1): 191-7. Epub 2010/09/08.

4. Hernandez R, Teruel T, Lorenzo M. Akt mediates insulin induction of glucose uptake and up-regulation of GLUT4 gene expression in brown adipocytes. FEBS letters. 2001; 494(3): 225-31. Epub 2001/04/20.

5. Ng Y, Ramm G, Burchfield JG, Coster AC, Stockli J, James DE. Cluster analysis of insulin action in adipocytes reveals a key role for Akt at the plasma membrane. The Journal of biological chemistry. 2010; 285(4): 2245-57. Epub 2009/11/10.

6. Riley JK, Carayannopoulos MO, Wyman AH, Chi M, Moley KH. Phosphatidylinositol 3-kinase activity is critical for glucose metabolism and embryo survival in murine blastocysts. The Journal of biological chemistry. 2006; 281(9): 6010-9. Epub 2005/11/08.

7. Smith U, Carvalho E, Mosialou E, Beguinot F, Formisano P, Rondinone C. PKB inhibition prevents the stimulatory effect of insulin on glucose transport and protein translocation but not the antilipolytic effect in rat adipocytes. Biochemical and biophysical research communications. 2000; 268(2): 315-20. Epub 2000/02/19.

8. Salinas M, Lopez-Valdaliso R, Martin D, Alvarez A, Cuadrado A. Inhibition of PKB/Akt1 by C2-ceramide involves activation of ceramide-activated protein phosphatase in PC12 cells. Molecular and cellular neurosciences. 2000; 15(2): 156-69. Epub 2000/02/16.

9. Martin SS, Haruta T, Morris AJ, Klippel A, Williams LT, Olefsky JM. Activated phosphatidylinositol 3-kinase is sufficient to mediate actin rearrangement and GLUT4 translocation in 3T3-L1 adipocytes. The Journal of biological chemistry. 1996; 271(30): 17605-8. Epub 1996/07/26.

10. Cheatham B, Vlahos CJ, Cheatham L, Wang L, Blenis J, Kahn CR. Phosphatidylinositol 3-kinase activation is required for insulin stimulation of

pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. Molecular and cellular biology. 1994; 14(7): 4902-11. Epub 1994/07/01.

11. Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. The Journal of biological chemistry. 1996; 271(49): 31372-8. Epub 1996/12/06.

12. Berger J, Biswas C, Vicario PP, Strout HV, Saperstein R, Pilch PF. Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting. Nature. 1989; 340(6228): 70-2. Epub 1989/07/06.

13. Flores-Riveros JR, McLenithan JC, Ezaki O, Lane MD. Insulin down-regulates expression of the insulin-responsive glucose transporter (GLUT4) gene: effects on transcription and mRNA turnover. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90(2): 512-6. Epub 1993/01/15.

14. Hajduch E, Hainault I, Meunier C, Jardel C, Hainque B, Guerre-Millo M, et al. Regulation of glucose transporters in cultured rat adipocytes: synergistic effect of insulin and dexamethasone on GLUT4 gene expression through promoter activation. Endocrinology. 1995; 136(11): 4782-9. Epub 1995/11/01.

15. Petersen S, Bahr M, Eckel J. Insulin-dependent regulation of Glut4 gene expression in ventricular cardiomyocytes: evidence for a direct effect on Glut4 transcription. Biochemical and biophysical research communications. 1995; 213(2): 533-40. Epub 1995/08/15.

16. Antonescu CN, Huang C, Niu W, Liu Z, Eyers PA, Heidenreich KA, et al. Reduction of insulin-stimulated glucose uptake in L6 myotubes by the protein kinase inhibitor SB203580 is independent of p38MAPK activity. Endocrinology. 2005; 146(9): 3773-81. Epub 2005/06/11.