Insulin and cellular stress induced glucose uptake in 3T3-L1 adipocytes

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Summary.

The research on 3T3-L1 adipocytes described in this thesis demonstrates how two different types of cellular stress inducing agents, namely the vicinal thiol binding agent arsenite and the conventional PKC-binding and -activating agent PMA act to increase glucose uptake in these cells. Whereas arsenite uses mainly the insulin-responsive GLUT4 transporter, PMA increases basal glucose transport through the GLUT1 transporter. As described in Chapter 3, arsenite-induced glucose uptake illustrates several requirements needed by any agent acting through GLUT4. These are, a tyrosine kinase activity, p38 MAPK activation and PKC-λ activity. Though PI-3' kinase activation is an essential step in insulin-signalling, this step is not required for arsenite-induced glucose uptake. Apparently, the need for tyrosine-kinase activity in arsenite induced glucose uptake resides in the ability to tyrosine-phosphorylate Cbl (see Chapter 3 Fig. 5). A further illustration of the importance of Cbl-tyrosine phosphorylation comes from our studies on rottlerin (Chapter 4). The ATP-depletion mediated by this pharmacological compound does not seem to be responsible for the observed inhibition of GLUT4 translocation (as was postulated by Kayali *et al.*[1]). Rather, aside from acting as an uncompetitive inhibitor of GLUT4, rottlerin hampers Cbl tyrosine phosphorylation, which leads to a 75% reduction in GLUT4 translocation (see Chapter 4, Fig. 3 and 4).

Regrettably, the nature of the arsenite-induced tyrosine-kinase activity remains as of yet unidentified. Though the specific ability of arsenite to induce STAT5a tyrosine-phosphorylation in the mature adipocyte, should provide a straightforward tool to enable its identification (J.L González-Galindo, unpublished observations)

Previously it had been demonstrated that insulin-induced p38 MAPK was involved in regulating the amount of glucose taken up by the cell without affecting GLUT4 translocation, suggesting some kind of intrinsic effect on the GLUT4 transporter itself [2]. Our observations on arsenite, a potent activator of p38 MAPK, illustrate a similar phenomenon in GLUT4-mediated stress-induced glucose uptake (see Chapter 3, Fig. 6). Subsequent research, described in a recently submitted manuscript, provides a detailed analysis of the involvement of p38 MAPK. These data demonstrate that p38 MAPK is involved in fine-tuning glucose uptake by regulating the turnover capacity of the GLUT4 transporter. A further note on the fine tuning of GLUT4-mediated glucose uptake comes from the observations on genistein, described in Chapter 5. This research suggests that in GLUT4 the turnover capacity for glucose can also be regulated through an intracellular ATP-binding Walker B motif akin to that described for GLUT1 [3]. Though further research is required to elucidate

this mechanism, this theoretical resolution constitutes a significant step forwards towards understanding mechanisms in action after GLUT4 membrane translocation. If these observations are mechanistically linked in the cell remains to be elucidated.

Aside from leading to enquiries into the mechanisms of insulin-induced glucose uptake, arsenite also opened up an avenue of more physiological research. We observed that arsenite-induced glucose uptake was sensitive to treatment with the insulin-resistance inducing agent dexamethasone. Subsequent analysis (described in Chapter 7) learned that although PI-3' kinase signalling is affected, in 3T3-L1 adipocytes the signalling pathway downstream is able to absorb this impediment. Rather, MKP-1 and -4 are upregulated in response to dexamethasone. Consequentially p38 MAPK activity is lost, leading to a reduction in glucose uptake. Given that MKP-4 is also upregulated in db/db- and ob/ob-mice [4], and that treatment of db/db mice with a glucocorticoid-receptor antagonist improves blood glucose levels [5;6], attenuation of p38 MAPK-signalling could be an important factor in type II diabetes.

To enable the studies described in this chapter, a novel tool had to be developed. 3T3-L1 adipocytes have for long been inaccessible to ectopic expression of DNA. By the application of Lentivirus as described in Chapter 6, a large number of cells can be efficiently and reliably transduced. This novel tool will make the 3T3-L1 adipocyte readily amendable to routine molecular biological techniques, which will be of great benefit to the research field.

In contrast to arsenite, PMA does not induce GLUT4 translocation, but acts solely through GLUT1. As illustrated in Chapter 8 of this thesis, in 3T3-L1 adipocytes the earliest and most PMA-sensitive PKC isoform is PKC-βII. But rather than activation, it is the concomitant degradation of this isoform which induces GLUT1 translocation. Further research (described in Chapter 9) highlighted the processes involved : First transcription of GLUT1, operating through the classical PKC-ERK-GLUT1 pathway. Second, translocation of GLUT1. This translocation is mediated by PKC-λ, which is found associated with PKC-βII in the basal state. Thus upon degradation of the βII-isoform (or disruption of this complex by treatment with myristoylated pseudo-substrate peptides against βII) PKC-λ is released and free to act as a positional queue inducing translocation of the GLUT1 containing vesicles.

References

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