

**FUNCTIONAL CHARACTERIZATION AND PHYSIOLOGICAL
REGULATION OF A GLUT4 HOMOLOG IN SALMONIDS**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

In most animal cells, glucose is transported across cell membranes by facilitated diffusion mediated by a family of glucose transporters (GLUTs). Several GLUT isoforms (GLUT1-5, 8-11) have been identified in mammals, with different tissue-specific expression patterns and biochemical properties. In particular, GLUT4 is expressed predominantly in muscle and adipose tissue and is responsible for the stimulation of glucose uptake in these two tissues by insulin. In mammals, insulin rapidly increases the number of GLUT4 molecules at the plasma membrane through its stimulation of the translocation of GLUT4 from intracellular storage sites in both muscle and adipose cells (Kahn 1996). In fish, insulin is also known to increase the in vivo uptake and utilization of glucose in skeletal muscle and to regulate the number of specific insulin receptors in muscle as well as in adipose tissue. Although the direct effects of insulin on glucose uptake by fish muscle or adipose cells are not clearly established, it is possible that insulin could exert its hypoglycemic effect by stimulating glucose uptake mediated by an insulin-sensitive GLUT. In order to address this question, we initiated studies to identify the presence of a GLUT4 homolog in fish (salmonid) muscle and adipose tissue and study its function and regulation.

Identification of a GLUT4 homolog in fish muscle and adipose tissue

Initially, we cloned by RT-PCR followed by 5'- and 3'-RACE a GLUT molecule from brown trout (*Salmon trutta*) skeletal muscle with high homology (approximately 80%) with mammalian GLUT4 sequences (Planas et al. 2000). This transporter, which was initially named btGLUT and which encoded for a protein of 503 amino acids, had structural features typical of a

functional GLUT (e.g. 12 transmembrane domains, intracellular amino- and carboxi-termini, one large intracellular loop between TMVI and VII, etc.). In addition, btGLUT showed several GLUT4-specific motifs, known to be important for trafficking in mammalian cells. btGLUT was found to be predominantly expressed in skeletal muscle, kidney and gill, which are tissues with high glucose utilization, and to a lesser extent also in adipose tissue, testis and intestine. Subsequently, we have cloned another GLUT4 homolog by screening a coho salmon (*Oncorhynchus kisutch*) adipose cDNA library with a btGLUT cDNA probe. This molecule has high homology to mammalian GLUT4 (again 80%) and is almost identical (over 95% identity) to btGLUT, indicating that okGLUT4 and btGLUT correspond most likely to the same isoform of transporter (GLUT4) in adipose tissue and muscle, respectively, from two different salmonid species

Functional characterization of the fish GLUT4 homolog

In order to study the glucose transport properties of the fish GLUT4 homolog, we have expressed okGLUT4 in *Xenopus* oocytes and have determined its kinetic and biochemical characteristics. The K_m or affinity constant for 2-deoxyglucose (2-DG) transport was in the range of 5 to 9 mM measured under zero-*trans* conditions. These values were higher than the value found for GLUT4 in mammals (5 mM) but lower than those for rainbow trout GLUT1 expressed in the same system (8-15 mM) (Teerijoki et al. 2001). Transport of 2-DG by okGLUT4-injected oocytes was stereospecific, and was competed by D-glucose, D-mannose and, to a lower extent, D-galactose and D-fructose. 2-DG transport was inhibitable by cytochalasin B and ethylidene glucose in a concentration-dependent manner. Moreover, glucose transport in oocytes expressing okGLUT4 was significantly stimulated by insulin.

Regulation of btGLUT expression by blood insulin levels

In order to study the possible physiological regulation of the expression of btGLUT in fish, we have examined by Northern blot its expression in red and white skeletal muscle from trout in which blood insulin levels had been manipulated experimentally. The expression of btGLUT in red muscle increased significantly when insulin plasma levels were elevated either by insulin (10 $\mu\text{g}/100\text{g}$ fish; 24 hours; Fig. 1) or arginine (6.6. $\mu\text{mol}/\text{g}$ fish; 24 hours) treatment and decreased significantly when insulin plasma levels were reduced either by fasting (45 days) or by feeding a low-protein, high-carbohydrate diet (2 months). In contrast, the expression of btGLUT in white muscle was not affected by changes in the plasma levels of insulin. Contrary to btGLUT expression, no changes in the expression of OnmyGLUT1, a

rainbow trout GLUT1 homologue, were detected in either red or white muscle in response to alterations in insulin plasma levels. These results strongly suggest that insulin could be regulating the expression of btGLUT in trout red muscle in vivo. It will be important to determine if insulin increases btGLUT mRNA levels because of an increase in the rate of transcription of the btGLUT gene.

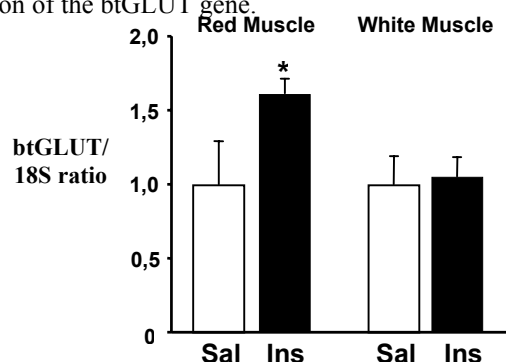


Figure 1. Effects of insulin treatment on btGLUT mRNA expression in red and white muscle. Sal, saline; Ins, insulin.

Conclusions

Our results indicate that the muscle and adipose tissue of salmonid fish express a structural and functional GLUT4 homolog. We have shown that insulin can stimulate its expression in red skeletal muscle and can cause a modest increase in glucose uptake when the transporter is expressed in *Xenopus* oocytes. However, the fish GLUT4 homolog has lower affinity for glucose than mammalian GLUT4, which could help explain the lower ability of fish to clear a glucose load, when compared to mammals. Key to our understanding to the function of the fish GLUT4 homolog will be to determine its ability to be recruited to the plasma membrane in response to insulin.

References

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