Physiological role of aquaporin 8 in adipocytes : molecular biological and live cell imaging approach with 3T3-L1 cells.

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Abstract

Introduction

Aquaporin-8 (AQP8), a member of the aquaporin water channel family, is expressed in various tissue and cells, including liver, testis, and pancreas. AQP8 appears to have functions on the plasma membrane and/or on the mitochondrial inner membrane. Mitochondrial AQP8 with permeability for H_2O, H_2O_2 and NH_3 has been expected to have important role in various cells, but its information is limited to a few tissues and cells including liver and kidney.

In this study, we found that, in mouse adipose tissues and 3T3-L1 preadipocytes, the mitochondrial type AQP8 is dominantly expressed. We therefore created an AQP8-knocked down cell line in which we have investigated the role of AQP8 on mitochondrial function.

Results and Discussion

1. Expression and localization of AQP-8 in adipose tissue and 3T3-L1 cells.

Cellular localization of AQP8 was examined by western blotting and immunocytochemistry. AQP8 was expressed in mouse adipose tissues and in preadipocyte-derived 3T3-L1 cells, and was localized to the mitochondria.

2. Effect of AQP8-knockdown on mitochondrial function

To investigate the function of AQP8 in the mitochondria, we transfected the 3T3-L1 cells with a plasmid coding shRNA designed to knockdown AQP8. In shAQP8 treated cells (shAQP8 cells), mRNA and protein levels of AQP8 were successfully decreased by about 75%. The rate of oxygen consumption was decreased and the cellular ATP level was significantly reduced in the shAQP8 cells. The activities of two enzymes which produce water as a byproduct, cytochrome c oxidase and ATP synthase were significantly reduced by AQP8 knockdown. In shAQP8 cells, marked swelling of the mitochondria was observed by electron microscopy, but the viability of cells was maintained.

These observations lead us to the hypothesis that the reduced AQP8 expression disrupts normal mitochondrial water flux, and lowers electron transport activity and ATP synthesis through inhibition of the water-generating processes.

We also found that knockdown of AQP8 had multiple effects on the cellular metabolism including reduction of fat accumulation and changes in glycolysis and lipolysis. Thus, AQP8 appears to functionally support cellular metabolism through maintenance of mitochondrial function.

3. Effect of energy metabolism on AQP8 expression

To examine the relationship between mitochondrial metabolic status and AQP8 gene expression, we focused on the AMPK (AMP-activated protein kinase) signaling, which is activated by increase in the cellar AMP:ATP ratio and plays an important role in the acceleration of energy metabolism.

In 3T3-L1 cells, the expression of AQP8 mRNA increased on treatment with AICAR, an AMPK activator, in a concentration-dependent manner. The up-regulation of AQP8 mRNA levels by AICAR was inhibited by adding compound C, an inhibitor of AMPK activation. Moreover, the knockdown of the NAD-dependent deacetylase sirtuin1 (SIRT1) with siRNA suppressed AQP8 gene expression.

The observed correlation between the expression levels of proteins responsible for mitochondrial energy metabolism and AQP8 implies that AQP8 expression is regulated to meet the demand of the enzymatic processes.

Conclusion

We found that AQP8 is expressed in the mitochondria in mouse adipose tissues and 3T3-L1 preadipocytes. Reduction in AQP8 expression disrupts normal mitochondrial water flux, and lowers electron transport activity through inhibition of the water-generating processes. Moreover, mitochondrial AQP8 gene expression is correlated with the proteins for mitochondrial respirations. These results suggest that mitochondrial AQP8 functionally supports mitochondrial respiration probably via water excretion.