Karolinska Institutet

Institution för molekylär medicin och kirurgi

Molecular Regulators of Glucose and Lipid Metabolism in Skeletal Muscle

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Farmakologens föreläsningssal, Nanna Svartzväg 2

Måndagen den 21 maj, 2012, kl 13.00

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Stockholm 2012

ABSTRACT

Skeletal muscle is a primary site of insulin action and insulin-stimulated glucose transport and occupies a center stage in maintaining whole body glucose and lipid homeostasis. Another key feature of a healthy skeletal muscle is its ability to switch between utilization of lipids and glucose as fuel in response to feeding or fasting respectively. This metabolic flexibility is impaired in skeletal muscle from insulin resistant and type 2 diabetic patients. Key molecules such as AMP Kinase [AMPK] and Pyruvate Dehydrogenase Kinase [PDK] play a crucial role in maintaining metabolic flexibility in a healthy skeletal muscle. These molecules have recently emerged as potential drug targets to combat diabetes.

One strategy to activate the AMPK pathway is via altering the expression of AMP-metabolizing enzymes, such as 5'-nucleotidases [NT5C]. The role of cytosolic 5′-nucleotidases was determined in metabolic responses linked to the development of insulin resistance in obesity and type 2 diabetes (Study I). NT5C1A and NT5C2 gene silencing led to increase in the AMP/ATP ratio, increase in phosphorylation of AMPK and ACC, and an increase in palmitate oxidation and glucose transport in mouse and human skeletal muscle.

Another strategy to activate the AMPK system would be to lower the threshold of AMPK activation by rendering AMPK more sensitive to its activators. This strategy has been undertaken in this thesis by pre-treating skeletal muscle with methotrexate [MTX] and targeting 5-Amino-4-imidazolecarboxamide Ribonucleotide Transformylase [ATIC] (Study II). MTX is an inhibitor of ATIC, an enzyme involved in *de-novo* nucleotide biosynthesis. ATIC imposes a metabolic block leading to intracellular ZMP accumulation, lowering the threshold for AMPK activation.

Increased glucocorticoid action leads to reduced whole body insulin action and may predispose to type 2 diabetes. Local conversion of cortisone to active cortisol by the enzyme 11β-hydroxysteriod dehydrogenase [11β-HSD1] in target tissues may regulate tissue specific roles of glucocortioids in pathophysiological states. Chronic high dose exposure to cortisone or cortisol reduced glucose metabolism and enhanced lipid metabolism, via induction of Pyruvate dehydrogenase kinase 4 [PDK4] expression in myotubes. siRNA-mediated reduction or pharmacological inhibition of 11β-HSD1 prevented the effects of cortisone but not cortisol, on metabolic responses (Study III).

Type 2 diabetes mellitus is associated with abnormal substrate metabolism, raising the possibility that alterations in the expression of mitochondrial enzymes controlling lipid uptake and metabolism may be altered (Study IV). Evidence that expression of key enzymes regulating mitochondrial function in skeletal muscle is altered in type 2 diabetes mellitus [T2DM] patients is provided.

In summary, activation of AMPK can play a central role in overcoming impairments in skeletal muscle glucose and lipid metabolism and this can be achieved by perturbing the enzymes involved in nucleotide metabolism such as 5'-nucleotidases and ATIC. Alterations in molecular regulators of substrate metabolism such as PDK4, reflect pathogenic condition and could be targeted to restore skeletal muscle energy homeostasis.