EDITORIAL FOCUS

Rethinking the regulation of L-carnitine transport in skeletal muscle cells.
Focus on “Multiple AMPK activators inhibit L-carnitine uptake in C2C12 skeletal muscle myotubes”

Da Xu and Guofeng You
Department of Pharmaceutics, Rutgers University, Piscataway, New Jersey
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CARNITINE IS A CRITICAL COFACTOR in the metabolism of lipids and therefore in the production of cellular energy. L-Carnitine, the active form, plays an important role in oxidizing fatty acids, transporting long chain fatty acids across mitochondrial membrane, and modulating intracellular coenzyme A homeostasis (3). L-Carnitine uptake into cells is mediated primarily by the organic cation/carnitine transporters (OCTN), a subclass of the solute carrier 22 transporter family. Patients bearing mutations in OCTN2 gene exhibit severe symptoms because of the resulting cardiomyopathy, progressive skeletal weakness, nonketotic hyperglycemia, and hyperammonemia (10). Currently the information on the regulation of L-carnitine transport into skeletal muscle cells is sparse. AMP-activated protein kinase (AMPK), a cellular energy sensor, usually acts to inhibit activation and L-carnitine transport is novel, because it challenges the common notion that AMPK, as a cellular energy guardian, would promote the uptake of L-carnitine, a critical player in fatty acid metabolism and cellular energy creation. This study provides fresh insights into the pharmacological treatment of L-carnitine deficiency for the improvement of L-carnitine accumulation in skeletal muscle to increase metabolism.

Indeed, the opposite linkage between AMPK activation and L-carnitine transport, discovered by Shaw and colleagues (8), is intriguing, and is the first step into a fascinating area of investigation. Many questions remain to be answered: 1) Although the authors detected the expression of OCTN1/2/3 in C2C12 cells, it would be imperative to address whether AMPK indeed regulates L-carnitine uptake through these transporters. If so, which specific OCTN isoform is involved? It should be noted that transporters other than OCTN1/2/3 could also transport L-carnitine, including OCT6 and amino acid transport system B0,+ (ATB 0,+) (10). 2) Once the specific carnitine transporter is identified and the involvement of AMPK is confirmed, it would be interesting to explore the mechanism underlying the regulation by AMPK. AMPK may affect transporter activity by altering the binding affinity of the transporter to its substrates, by altering the expression, membrane trafficking, and stability of the transporter, or by altering the interaction of the transporter with its interacting partners (7). 3) The AMPK activators used in this study (except caffeine, which exerts it role, at least in part, through AMPK) may function to bypass AMPK and act directly on the transporters as competitive or noncompetitive inhibitors, leading to the inhibition of L-carnitine transport. Thus, it would be important to assess this possibility. 4) Although the inhibitory effect of AMPK on L-carnitine transport in C2C12 cells contrasted with what was expected of a stimulatory effect, the opposite regulation by AMPK on the membrane transporter has been observed in other tissues, contingent on the timing and chronicity of AMPK activation. For example, acute activation of AMPK causes a downregulation of Na+-dependent glucose transporter SGLT1 in gut (5), whereas chronic activation of AMPK in transgenic mice bearing a gain-of-function mutation in AMPK causes an enhanced SGLT1 activity in heart (1). Therefore, the behavior of AMPK in C2C12 cells may bear such analogy. 5) AMPK is a heterotrimeric kinase consisting of one catalytic α-subunit and two regulatory β- and γ-subunits. Although it has been acknowledged that each subunit exists in multiple isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) with various expression level and tissue distribution, the exact function of these isoforms remains unknown. For example, human heart is reported to have a large amount of α1-AMPK activity, whereas α2-AMPK...
accounts for a majority role in mouse heart (4). Since the current study is using a mouse myoblast cell line, it is possible that the difference in the composition of AMPK isoforms between human and rodent could cause the unexpected effect on L-carnitine uptake. Finally, it should also be kept in mind that, depending on the cell type, protein-protein interactions, signaling pathways, and the level of expression of the individual transporter can be quite different. Therefore, it is crucial to confirm and extend the current findings from C2C12 cells (8) in another cell line, or in primary cells, or even in vivo so that the regulation of L-carnitine transport in muscle cells can be thoroughly understood.

Taken together, to our knowledge, Shaw and colleagues have pioneered in the field of AMPK regulation of L-carnitine transport in skeletal muscle cells. Further exploration in this area could lead to thrilling implications in our understanding of L-carnitine transport processes in muscle cells as well as in other tissues and provide pharmacological basis for the treatment of L-carnitine deficiency.

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REFERENCES