MOLECULAR METABOLISM

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A tale of two methods: Identifying neuronal CB_1 receptors

A paper by Dr. G. Marsicano's group [1] in this issue of Molecular Metabolism is clarifying and further elaborating on some of their previous results on the action of cannabinoid type 1 receptor (CB_1) in neuronal mitochondria [2]. This new article was written, at least in part, in response to our finding that anti-CB_1 serum used in this as well as in other studies also recognizes mitochondrial stomatin-like protein 2 [3]. Although we did not specifically criticize their major findings and general approach, we did emphasize the possible technical issues that can occur when these antibodies are used.

We believe that it is a constructive course of action when different laboratories publicly discuss limitations of methodologies and reliability of the data interpretation in biomedicine. Study of mitochondria in the brain is one of those topics that merit such discourse. "Hard core" mitochondrial bioenergetics has been most extensively studied in tissues with homogenous cell populations, such as the liver, muscle, and heart, that most reliably (albeit not perfectly) provide isolation of mitochondria from the cell type of interest (e.g., hepatocytes and myocytes). The brain, however, consists of multiple cell types and subclasses, and it is virtually impossible to isolate very pure mitochondrial fractions using currently available technologies. In accord, Marsciano and colleagues previously reported that the CB_1 agonist, WIN 55,212-2 (WIN), at concentrations of 50 nM and 100 nM, reduced mitochondrial respiration by about 40% and 50%, respectively [2]. In the new study, they report approximately 7% and 20% reduction of mitochondrial respiration at corresponding concentrations of WIN [1]. A confounding factor may be contamination of mitochondrial fractions with the fragments of axonal cell membranes that are known to contain CB_1. Hebert-Chatelain et al. [1] also used more sensitive immunoperoxidase cytochemical method with DAB—Ni as a chromogen. They now report mitochondrial labeling in mice of different genotypes, including CB_1-KO mice, similar to what we reported.

We agree with Dr. Marsicano that adapted experimental procedures and proper controls are essential for correct interpretations of the data. Their and our observations are not too dissimilar; nevertheless, different conclusions may be drawn. We hope that these constructive interactions will increase the drive to improve currently available experimental technologies so that fundamental issues regarding brain mitochondria can be more conclusively addressed in future studies.

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