MOLECULAR METABOLISM

Correspondence

Studying mitochondrial CB1 receptors: Yes we can

"For those who believe, no proof is necessary. For those who don't believe, no proof is possible"

(Stuart Chase, American engineer and economist, 1888–1985)

In biology, particularly in neuroscience, confirmatory studies are always easier to accept than new ideas. Of course, the onus of providing evidence supporting new hypotheses belongs to the individuals who propose them. The existence of mitochondrial CB1 receptors (mtCB1) belongs to this type of hypothesis.

In 2008, when we first observed the presence of CB1 receptors at brain mitochondrial membranes, we were aware of the unconventionality of this finding. Thus, we armed ourselves with patience and used everything in our power to challenge this idea. To this aim, all our experiments were performed using the best available negative controls [1], which are mice genetically modified to lack the entire coding sequence of the *CB1* receptor gene (*CB1*-KO) [2]. For instance, only after verifying that careful and reproducible quantifications displayed significantly higher levels of immunogold staining of brain mitochondria with CB1 antisera in wild-type mice as compared with *CB1*-KO littermates, we concluded that some expressions of mtCB1 likely exist in the brain [1]. While all antisera display some levels of unspecific binding to extracts and tissues, the use and quantification of negative controls is the only procedure that allows for determining antisera specificity. In agreement with Morozov et al. [3], we now report [4] that the DAB–Ni technique produces higher background mitochondrial staining than the immunogold approach originally used [1], likely because mitochondria contain biotinylated proteins [5,6]. Still, careful quantifications revealed a significantly higher staining of brain mitochondria in wild-type than in *CB1*-KO tissues, independently of the method used [1,4].

In their commentary [7], Morozov et al. underline the difficulty of brain mitochondrial purifications and correctly highlight some discrepancies in the amplitudes of cannabinoid effects on mitochondrial respiration between our two studies [1,4]. However, while brain mitochondrial purification is difficult, we maintain that when a drug repeatedly produces a significant effect on wild-type extracts and no effects on extracts from *null* mutants for a specific protein, one can conclude that this protein mediates the observed effect, independently of its amplitude. Moreover, the data presented in this issue of *Molecular Metabolism* suggest that the effects of WIN55,212-2 are decreased by higher synaptosomal contaminations (see Figs 4 and 5 of Ref. [4]), indicating that lower purification quality likely decreases cannabinoid impact on mitochondrial respiration, rather than the contrary, as implicitly proposed by Morozov et al. [3,7].

Only the use of additional controlled experimental approaches providing quantifiable data will confirm the existence, or not, of mtCB1 receptors. Given the currently available evidence, however, we think that the direct impact of (endo)cannabinoid signaling on brain mitochondrial functions represents a novel and reasonable possibility to explain some of the mechanisms of brain functions, which will be worth exploring further.

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Etienne Hebert-Chatelain[1,2], Leire Reguero[3], Nagore Puente[3], Beat Lutz[4], Francis Chaouloff[1,2], Rodrigue Rossignol[2,5], Pier-Vincenzo Piazza[1,2], Giovanni Benard[1,2], Pedro Grandes[3,**], Giovanni Marsicano[1,2,*]

[1] *INSERM U862, NeuroCentre Magendie, 33077 Bordeaux, France*

[2] *University of Bordeaux, 33077 Bordeaux, France*

[3] *Department of Neurosciences, Faculty of Medicine and Dentistry, University of the Basque Country UPV/EHU, 48940 Leioa, Spain*

[4] *Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany*

[5] *Laboratoire Maladies Rares: Génétique et Métabolisme, 33077 Bordeaux, France*

*Corresponding author. INSERM U862, NeuroCentre Magendie, 33077 Bordeaux, France.

** Corresponding author.

E-mail addresses: onpgrmop@lg.ehu.es (P. Grandes), giovanni.marsicano@inserm.fr (G. Marsicano)

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