Commentary

MOLECULAR METABOLISM

A mighty (ochondrial) fight?

Daniele Piomelli [1,2,3,*]

Disputes about scientific findings unfold following a well-rehearsed script. One researcher (let's call her Dr. X) makes an intriguing new observation and manages to get it past the refereeing process of a top journal. The study is published and Dr. X happily moves on to her next project. But she is interrupted by an annoying piece of news — a report from the lab of another scientist (let's call him Dr. Y) flatly contradicts her. Making things worse, Dr. Y is a real expert in the particular set of techniques used by Dr. X, so much so that his opinion cannot be simply tossed away as irrelevant. But Dr. X believes in her data, so she sets out to vindicate them. She runs new, even more stringent tests and replicates successfully her previous work. Will the new experiments convince Dr. Y? Probably not (unless, of course, he was directly involved in running them). And what about other scientists? Will *they* be convinced? Friends and family will probably take sides, but the majority will more wisely wait for the dust to settle. If Dr. X stands by her story and others reproduce her findings, the field will accept them and the debate will be eventually forgotten. The wait can be uncomfortable, especially for the parties involved, but this is how science normally works — scientists tend to adopt theories by making conscious or (more often) unconscious statistical assessments of the overall evidence available to them.

An article [1] and two back-to-back commentaries published in the present issue of Molecular Metabolism present an interesting experimental controversy, which will likely follow the standard course outlined above. The apple of discord was tossed two years ago by the unexpected finding, made in the laboratory of Giovanni Marsicano at the INSERM in Bordeaux, that CB_1-type cannabinoid receptors may be present on the membranes of neuronal mitochondria, and that pharmacological activation of these receptors may decrease the conversion of biochemical energy from nutrients into ATP [2]. This result was surprising enough to warrant a *News and Views* piece aptly titled 'Do cannabinoids reduce brain power?' [3]. But why the surprise? Because the standard view of CB_1 receptors is that they are localized, like other G protein-coupled receptors, to the surface of neuronal and glial cells, where they wait for exogenous chemicals (like Δ^9-tetrahydrocannabinol in marijuana) or endogenous neurotransmitters (like anandamide and 2-arachidonoylglycerol) to activate them. According to this view, the mitochondrion is not a proper place for a cannabinoid receptor to be hanging around. Yet, Marsicano and collaborators felt that they had at least one critical piece of evidence backing their unconventional claim: in their experiments, the mitochondrial staining for CB_1 receptor, assessed by an electron microscopy technique known as immunogold labeling, was substantially higher in wild-type mice than in mutant mice lacking the receptor. Most researchers would agree that this was, indeed, a decisive argument. The dispute started when the laboratories of two noted Yale neurobiologists, Pasko Rakic and Tamas Horvath, found that the CB_1 antibody utilized by Marsicano and coworkers also stained an unrelated mitochondrial protein, which they identified as stomatin-like protein 2 using a combination of immunoprecipitation and high-resolution mass spectrometry [4]. The staining was specific, in that it was abolished by pre-absorption with the peptide used to generate the CB_1 antibody, but was still visible on neuronal mitochondria of CB_1-deficient mice. In addition, dealing another blow to the mitochondrial CB_1 hypothesis, Rakic and Horvath did not see any functional effect of CB_1 receptor activation in mitochondria purified from mouse brain [4]. Nevertheless, the Yale researchers mitigated the strength of their conclusions by emphasizing the serious hurdles encountered in the isolation of purified mitochondria from brain tissue (as opposed to other more homogeneous organs such as liver and muscle). This technical difficulty creates a source of uncontrolled variability, which might well be an important part of the problem. For example, in the paper published in the present issue of Molecular Metabolism [1], Marsicano and collaborators were able to replicate their original results, but found that the CB_1 receptor agonist Win-55212-2 reduces mitochondrial respiration only by 7% and 20% at the concentrations of 50 nM and 100 nM, respectively, whereas in the original study values for inhibition at the same agonist concentrations were substantially higher (40% and 50%, respectively) [2]. Another, potential source of complexity is the fact that endocannabinoid substances such as anandamide and 2-arachidonoylglycerol differ in many ways from other neural messengers — being produced on demand and lipid-soluble [5], they might act both as transcellular and intracellular signals. It is reasonable to speculate that mitochondrial CB_1 might be an intracellular target for their action.

So, what's next? The parties involved in this intriguing discussion have done the right thing: they started a public (and civilized) conversation on the validity and interpretation of their findings. Now it's time for other scientists inside and outside the cannabinoid field to let new data speak up.

This commentary refers to "Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB_1 receptor by Hebert Chatelain et al.", http://dx.doi.org/10.1016/j.molmet.2014.03.007.

[1]Department of Anatomy and Neurobiology, University of California, Irvine, CA, USA [2]Department of Pharmacology and Biological Chemistry, University of California, Irvine, CA, USA [3]Drug Discovery and Development, Istituto Italiano di Tecnologia, Genova, Italy

*Department of Anatomy and Neurobiology, University of California, Gillespie Neuroscience Research Facility, Irvine, CA 92617-1275, USA. E-mail: piomelli@uci.edu.

Received April 11, 2014 • Accepted April 11, 2014 • Available online 18 April 2014

http://dx.doi.org/10.1016/j.molmet.2014.04.005

REFERENCES

[1] Hebert-Chatelain, E., et al., 2014. Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB_1 receptor. Molecular Metabolism 3:495–504.

[2] Benard, G., et al., 2012. Mitochondrial CB(1) receptors regulate neuronal energy metabolism. Nature Neuroscience 15:558–564.

[3] Alger, B.E., Tang, A.H., 2012. Do cannabinoids reduce brain power? Nature Neuroscience 15:499–501.

[4] Morozov, Y.M., Dominguez, M.H., Varela, L., Shanabrough, M., Koch, M., Horvath, T.L., et al., 2013. Antibodies to cannabinoid type 1 receptor co-react with stomatin-like protein 2 in mouse brain mitochondria. The European Journal of Neuroscience 38:2341–2348.

[5] Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. Nature Reviews Neuroscience 4:873–884.

