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Do cannabinoids reduce brain power?

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Abstract

Extracts from the Cannabis plants, cannabinoids, bind to the same receptors as do endogenous cannabinoids. Although usually found on nerve terminals where their activation inhibits transmitter release, cannabinoid receptors are reported by Bernard et al.¹ to exist on mitochondria, where their activation by endocannabinoids regulates energy metabolism.

The brain is a prodigious consumer of energy, accounting for 20% of the body's energy usage, yet only ~2% of its total weight (or, given the obesity epidemic, even less in many countries). The energy, in the form of ATP, is supplied by mitochondria. As a result, the brain is highly sensitive to mitochondrial dysfunction, and many disease or aging-related neurological disorders are associated with mitochondria². While commonly called the cell's power plants, mitochondria are also involved in a variety of other metabolism-related physiological functions, such as regulation of membrane potentials and Ca²⁺ signals that participate in synaptic plasticity. In recent years, the endocannabinoid system (ECS) has emerged as a ubiquitous and potent overseer of neuronal synaptic communication; now the Marsicano group demonstrates¹ that its influence extends to the control of neuronal energy metabolism.

The ECS comprises G-protein coupled cannabinoid receptors that bind to ligands typified by the psychoactive ingredient in *Cannabis*, ⁹Δ-tetrahydrocannabinol (THC), together with endogenous agonists – endocannabinoids, eCBs; mainly 2-arachidonyl glycerol (2-AG) and anandamide (AEA) – and their synthetic and degradative enzymes and transporters³. The main cannabinoid receptors of the brain, CB1s, are neuronal plasma membrane receptors (pmCB1s) usually found on or near presynaptic axon terminals, where they encounter eCBs that are synthesized and released from post-synaptic neurons. Once activated, pmCB1s initiate signaling cascades that inhibit synaptic transmitter release. The typical ECS actions thus represent a “retrograde” signaling process⁴.

When they were detected in the membranes of intracellular organelles⁵, it was assumed that CB1s were simply being transported to or from the cell body. The internal CB1s were not at first observed in mitochondria, and their C-termini were oriented towards the cytosol, as expected if they were destined for insertion into the plasma membrane via an exocytotic mechanism. In contrast, Bernard et al.² report that a large fraction (~15%) of all CB1s are found in mitochondrial outer membranes with their C-termini facing the interior of the organelle, consistent with a signaling function. Indeed 30% of neuronal mitochondria carry

CB1s (mtCB1s), and both THC and eCBs suppress complex I and respiration in isolated mitochondria, (see Figure), probably by reducing cAMP levels and PKA activity.

The contributions of mtCB1s and pmCB1s were teased apart, using novel methods, and all experiments were tightly controlled by comparisons between tissue from normal and CB1^{-/-} animals. Classical CB1 agonists and antagonists are lipophilic compounds that have equal access to internal and external receptors. The experimental strategy compared the actions of the classical drugs with those of membrane-impermeant ligands that could only bind to pmCB1s – the difference should reflect the contribution of mtCB1s. Indeed, in intact cells an impermeant CB1 peptide antagonist (inverse agonist), hemopressin⁶, fully blocked pmCB1 actions, while leaving the depression of mitochondrial respiration by membrane-permeant CB1 agonists unimpaired. Parallel tests with a novel membrane-impermeant CB1 agonist, HU-biot, made by attaching a biotin moiety to the membrane-permeant agonist, HU210, showed that unlike HU210, HU-biot did not reduce cellular respiration. Hemopressin and HU-biot were fully effective in permeabilized cells, so the results reflected differences in intracellular access and not in efficacy.

Evidently, exogenous cannabinoids can activate mtCB1s and regulate mitochondrial function. Can eCBs do the same? There are two ways to elevate eCB levels: enhancing eCB mobilization or inhibiting eCB degradation. While fatty-acid amide hydrolase (FAAH), the primary degradative enzyme for AEA, was known to be densely present in the mitochondria, Bernard et al.² found that purified mitochondria also have substantial 2-AG degrading activity attributable to monoacylglycerol lipase (MAGL). When MAGL was inhibited in purified mitochondria, their 2-AG levels were significantly increased and respiration was suppressed correspondingly – the higher the 2-AG amount, the lower the respiration. Hence, eCBs are generated in close association with mitochondria and they down-regulate respiration by activating mtCB1s. Convincing evidence thus ties mtCB1s to suppression of mitochondrial energy metabolism.

What happens in intact neurons? Depolarization-induced suppression of inhibition, DSI, is produced when depolarization of a post-synaptic cell opens voltage-gated Ca²⁺ channels, and thereby triggers synthesis and release of eCBs proportionate to the strength of the depolarization. DSI is a CB1-mediated depression of GABA release, and a well-established bioassay of eCB effects^{3,4}. In hippocampal slices, HU210 completely occluded strong DSI, while HU-biot only partially occluded it. Likewise, hemopressin partially reduced strong DSI, while the membrane-permeant CB1 antagonist, AM251, abolished it. Finally, a synthetic mitochondrial complex I inhibitor, rotenone, was ineffective on strong DSI, but enhanced weak DSI. The authors suggest that because rotenone suppressed mitochondrial function maximally, the component of strong DSI attributable to mtCB1s was occluded. Weak DSI normally did not involve mtCB1s and so it could be enhanced when rotenone reduced mitochondrial function. The conclusion was that, by suppressing mitochondrial complex I activity, mtCB1s potentiate the eCB actions, although the mechanism remains to be worked out. The predicted decrease in ATP could alter the energy supplies, Ca²⁺ levels, or redox potentials conducive to neurotransmitter release. Nevertheless, the main results supported the hypothesis: mtCB1s depress mitochondrial respiration and somehow enhance physiological, eCB-mediated responses.

Inevitably, exciting new findings like these are accompanied by some loose ends. Perhaps the most startling is that, in intact cells, direct pharmacological activation of mtCBs (i.e., not involving eCBs generated during DSI) does not influence synaptic transmission at all. This can be inferred from the observation that the agonists HU210 and HU-biot were equally effective in suppressing *basal* synaptic transmission. If HU210 acts on both pmCB1s and mtCB1s, it should have had a more pronounced effect than HU-biot, which affects only

pmCB1s. Along this line, hemopressin prevented the suppression of basal transmission by either HU210 or HU-biot, which apparently rules out a role for mtCB1s in this case. Finally, the finding that rotenone neither reduced basal GABA release nor potentiated the actions of HU-biot is most unexpected if the mtCB1-induced interference with mitochondrial function *per se* is the key step. Clearly, something is missing. Rotenone provides the only link between mtCB1s and eCBs, and the involvement of other internal CB1s, which could also have been affected by membrane-permeant ligands, is not ruled out. Alternatively, as the authors hint, it might be that the strong DSI mechanism requires more than simply the action of released eCBs on presynaptic CB1s. The postsynaptic depolarization and Ca^{2+} influx that initiates eCB mobilization for DSI might do other things as well, perhaps release another factor having detectable actions only when mitochondrial function is simultaneously reduced. (Could nitric oxide re-emerge as a player³?) The new results provide a rough outline of new things to come, rather than a detailed mechanistic diagram. Exogenous application of eCBs, or releasing them by postsynaptic uncaging of caged Ca^{2+} could help sort things out. Other tests could involve activation of metabotropic glutamate, acetylcholine, dopamine and a host of other GPCR-coupled receptors that release eCBs via relatively Ca^{2+} -insensitive (and depolarization-independent) mechanisms³.

Assuming that these issues can be resolved, what are some implications of the regulation of neuronal energy supplies by mtCB1s? An tantalizing lead is that mitochondria not only respond to eCBs, but can regulate, or even produce them, as well. Significant MAGL and 2-AG activities are detected in purified mitochondria and inhibiting the enzyme substantially increase 2-AG. Where does mitochondrial 2-AG come from, and what does it normally do? The major synthetic enzyme for 2-AG, DGL α , has not been reported to exist in neuronal mitochondria, but this could change; in any case, ~20% of 2-AG in the brain is independent of DGL α ^{7,8,9}, hence finding 2-AG in mitochondria may not be surprising. Whatever its source or normal function though, increases in mitochondrial 2-AG can activate mtCB1 and suppress cellular respiration, and this could be quite important.

Quantitative differences in CB1 activation can give rise to qualitative differences in eCB-mediated responses. For example, eCBs regulate synaptic transmitter release on both short and long time scales. DSI and similar phenomena last for seconds to minutes, but eCB-mediated long-term depression of synaptic transmission (eCB-LTD) is much more enduring. Although both short and long forms of eCB-mediated synaptic plasticity are induced by CB1s, DSI can be initiated within < 1 sec, whereas eCB-LTD induction requires CB1 activation persisting for several minutes¹⁰. PKA and cAMP are involved in the transition from short to long forms of eCB-dependent plasticity, though what factors actually control the transition have been obscure. Exploration of the role of mtCB1s in this process might prove rewarding.

Cannabis derivatives and the ECS are being investigated for their physiological effects and therapeutic potential in a variety of phenomena. An important tactic is to enhance the levels of eCBs, which are physiologically produced when and where needed, by interfering with their degradation¹¹. How will regulation of mitochondrial respiration by cannabinoids alter our interpretations of the problems and answers arising from these studies? Marsicano et al.¹² reported that neurotoxic damage inflicted by kainic acid is greater in mice lacking CB1 than in wild-type animals; evidently eCBs were generated and the resulting CB1 activation was neuroprotective. However, mitochondrial functional insufficiency contributes to the extent of stroke-induced neurological damage². It now appears that eCBs released during neurocytotoxic insult could also, by suppressing mitochondria, oppose the neuroprotective effect and exacerbate the lesion, with the ultimate outcome depending on a balance of the two tendencies. Therapeutic approaches that increase eCB availability by decreasing eCB degradation may not avoid such complexities.

Lastly, certain diseases or other maladies are accompanied by upregulation of the ECS – examples are behavioral stress¹³, Fragile X Syndrome^{14,15}, Parkinson's disease², and others. Mitochondrial dysfunction has been suspected as an etiopathological co-factor in many of these cases². If upregulation of eCBs downregulates mitochondrial respiration, then eCBs and mtCB1s could link certain disease states with mitochondrial dysfunction. These and other implications of the new work will surely energize future investigations of the ECS.

Acknowledgments

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Reference List

1. Bernard G, Massa F, Puente N, Lourenco J, Bellochio L, et al. *Nat Neurosci.* 2012; xx:x.
2. Mattson MP, Gleichmann M, Cheng A. *Neuron.* 2008; 60:748–766. [PubMed: 19081372]
3. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. *Physiol Rev.* 2009; 89:309–380. [PubMed: 19126760]
4. Alger BE. *Prog Neurobiol.* 2002; 68:247–286. [PubMed: 12498988]
5. Bodor AL, Katona I, Nyiri G, Mackie K, Ledent C, et al. *J Neurosci.* 2005; 25:6845–6856. [PubMed: 16033894]
6. Heimann AS, Gomes I, Dale CS, Pagano RL, Gupta A, et al. *Proc Natl Acad Sci U S A.* 2007; 104:20588–20593. [PubMed: 18077343]
7. Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, et al. *J Neurosci.* 2010; 30:2017–2024. [PubMed: 20147530]
8. Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, et al. *Neuron.* 2010; 65:320–327. [PubMed: 20159446]
9. Alger BE, Kim J. *Trends Neurosci.* 2011; 34:304–315. [PubMed: 21507493]
10. Chevaleyre V, Takahashi KA, Castillo PE. *Annu Rev Neurosci.* 2006; 29:37–76. [PubMed: 16776579]
11. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, et al. *Nat Chem Biol.* 2009; 5:37–44. [PubMed: 19029917]
12. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, et al. *Science.* 2003; 302:84–88. [PubMed: 14526074]
13. Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, et al. *J Neurosci.* 2011; 31:10506–10515. [PubMed: 21775596]
14. Zhang L, Alger BE. *J Neurosci.* 2010; 30:5724–5729. [PubMed: 20410124]
15. Maccarrone M, Rossi S, Bari M, DeChiara V, Rapino C, et al. *Neuropsychopharmacol.* 2010; 35:1500–1509.

Figure 1.

Model of pmCB1s and mtCB1s in presynaptic nerve terminals. Stimulation is represented by solid arrows and inhibition by dotted lines. Postsynaptic depolarization induces Ca^{2+} influx and the subsequent activation of DGLα results in 2-AG synthesis and release. 2-AG is degraded by MAGL in mitochondria and other presynaptic intracellular compartments. Weak DSI produces only enough 2-AG for pmCB1 activation; strong DSI generates enough to also activate mtCB1s. Stimulation of pmCB1 reduces transmitter release mainly by inhibiting presynaptic, voltage-gated Ca^{2+} channels (not shown). Stimulation of mtCB1 inhibits the cAMP-PKA pathway and thereby inhibits the activity of complex I in the electron transport chain. The resulting reduction of mitochondrial respiration triggers further inhibition of transmitter release through an unknown mechanism. Complex I can also be inhibited by rotenone. Membrane permeant agonists such as HU210 or antagonists such as AM251 gain access to both pmCB1s and mtCB1s. However, hemopressin, a membrane-impermeant CB1 antagonist, prevents eCB effects only partially because it inhibits only pmCB1s. Similarly, the membrane-impermeant CB1 agonist HU-biot activates only pmCB1s and so cannot completely occlude eCB-mediated effects if mtCB1s are involved.