Metabolic Messengers: Endocannabinoids

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Abstract

The endocannabinoid system is considered one of the most important modulatory networks in the whole organism. Research has yielded great insight on the mechanisms that link endocannabinoids and several peripheral and brain metabolic functions. Here, we provide a brief overview of the metabolic roles of endocannabinoids in tissue-, cellular- and subcellular-dependent mechanisms. We highlight past discoveries and future open questions with a special focus on CB1 receptor-dependent astroglial and mitochondrial metabolic processes.

1. Introduction

With its almost 5000-year documented use, *cannabis sativa* is one of the oldest cultivated plants, possibly the first bred for reasons independent from direct food production[1,2]. We can, however, presume that the first users soon realized that its consumption induces, amongst a plethora of other effects, strong hunger and desire to eat appetitive foods[3]. After the introduction of the plant into the Western pharmacopeia in the 1800s for the treatment of various conditions, modern science, starting from the middle of last century, provided key discoveries in the field (Figure 1). The description of the chemical structure of the active components of the plant (cannabinoids, like the psychoactive Δ^9-tetrahydrocannabinol, THC, and others)[4,5], of the cellular type-1 and type-2 cannabinoid receptors (CB1 and CB2)[6,7], and of the endogenous lipid ligands (endocannabinoids)[8-10] with the machinery for their synthesis and degradation[11] (Figure 1) are the historical milestone discoveries leading to the definition of the so-called endocannabinoid system (ECS)[12], which is a fundamental modulatory system in the whole organism. With just a little exaggeration, one could affirm that there is almost nothing that happens in the body, from skin physiology, to immune responses, to metabolic and mental processes, that would not involve the activation of endocannabinoid signaling.

As we will see more in detail below, the majority of these diverse functions underlie the important general role of the ECS in modulating body energy metabolism. This short review will address these aspects, particularly focusing on how the modulation of "cellular" metabolism represents a novel way through which endocannabinoids control brain and body functions, and their interactions. This is linked to the discovery of novel cellular and subcellular localizations of CB1 receptors, which can deeply impact cellular metabolic processes with functional implications at the organismal level.

The definition of the ECS is continuously changing, with the addition of novel players directly or indirectly involved in its functions. Due to space limitations, the list of cannabinoid receptors and the endogenous molecules modulating their activity provided in this article is necessarily limited and incomplete (See Refs. 2,13-15 for more exhaustive descriptions). CB1 and CB2 are seven-transmembrane G Protein-Coupled

Receptors (GPCRs) and they were first discovered as the cellular targets of exogenous plant-derived (THC) or synthetic cannabinoids[6,7]. This discovery implied the existence of endogenous ligands, which were soon identified as lipid derivatives of arachidonic acid and called endo-cannabinoids[8,9]. The most studied endocannabinoids are 2-arachidonoyl-glycerol (2AG) and anandamide (AEA), but new concepts have arisen that should be taken into consideration when dealing with endocannabinoid signaling: (i) 2AG and AEA could bind to other targets than cannabinoid receptors[14], implying that their tissue level changes do not always reflect an activation of the ECS in strict sense; (ii) peptide endogenous ligands (PepCans) can bind and modulate the activity of cannabinoid receptors and were proposed to belong to the endocannabinoid family[16,17]; (iii) the synthetic and catabolic pathways of lipid endocannabinoids are redundant and involve also other lipids, such as prostaglandins and others[18]; (iv) non-arachidonic acid-derived lipids were identified as endogenous allosteric regulators of cannabinoid receptor activity, such as the anti-inflammatory Lipoxin A4 (Ref. 19) or the steroid precursor Pregnenolone[20], making these molecules members *de facto* of the ECS.

2. Endocannabinoid signaling, a cornerstone for whole-body energy metabolism

Few attempts have been made to obtain a unified vision of the large spectrum of functions controlled by endocannabinoid signaling in animal physiology. One of the most convincing ideas in our opinion is that endogenous CB1 receptor signaling might represent a powerful system to adjust body metabolism towards the accumulation of energy reserves. As such, endocannabinoid signaling would exert a prototypical exostatic function (accumulation of reserves for future potential needs), as opposed to endostatic ones, through which the body responds to present needs (see Ref. 21 for an exhaustive discussion). Thus, amongst other functions, endocannabinoids can rewire behavior towards more food intake (particularly palatable and energy-rich)[22], favor the accumulation of fat in the adipose system[23], increase nutrients absorption in the intestinal tract[24], decrease glucose use in the muscles[25], increase lipogenesis in the liver[26] and increase insulin secretion in the pancreas[27] (Figure 2). In line with this, other roles of endocannabinoid signaling such as in the reduction of passive fear responses[28], of pain[29], of anxiety[30], or the gating of motivation for physical exercise[31-33] would help individuals to be resilient against adversities and to physically engage in the search and

consumption of food[21]. Similarly, endocannabinoids seem to enhance sensory perception, like vision, olfaction or taste[34,35], all conditions that would favor the detection of nutrients in the environment[21]. Thus, endocannabinoids play a key role in the control of body energy metabolism.

The link between endocannabinoid signaling and energy metabolism is based on an overwhelming amount of experimental and clinical data. In the second half of the years 2000, the CB1 receptor blocker rimonabant was commercialized as a therapeutic aid against obesity and associated metabolic disorders[36]. Before being withdrawn in 2008 because of undesired side effects linked to anxiety and depression[37], rimonabant showed its efficacy in reducing not only body weight, but also many other obesity-associated metabolic dysfunctions[36,38,39]. These aspects have been listed and discussed by several reviews over the years[3,40,41]. In this section, we will just shortly highlight how endocannabinoid signaling through CB1 receptors can impact central and peripheral mechanisms involved in energy metabolism. For space reasons, we will focus on CB1 receptor signaling, but it should be considered that the other main cannabinoid receptors (*i.e.,* CB2 receptors) have also been involved in whole-body metabolism[42,43].

Neuronal endocannabinoid signaling. CB1 receptors in the brain, especially those located in the hypothalamic areas, play a key role in controlling appetite and modulating certain neuropeptides to favor energy accumulation and storage[3,44]. In other brain regions involved in reward (i.e., ventral tegmental area or nucleus accumbens), the endocannabinoid signaling has also been linked to the control of hedonic feeding behavior[45,46]. As happen with other brain functions[47], depending on the brain regions where CB1 receptors are located, the control over food intake or energy balance can be very different. Indeed, this control also depends on whether CB1 receptors are located on specific cell types[22,48]. Thus, specific pools of CB1 receptors present in particular hypothalamic cells, such as the POMC neurons, or in the sympathetic system could modulate food intake[3,44,48]. Accordingly, different doses of (endo)cannabinoids could preferentially act on glutamatergic or GABAergic CB1 receptors to respectively increase or decrease food intake in mice[22]. Similar biphasic effects of cannabis on food consumption were also observed in humans[49].

Endocannabinoid signaling in adipose tissue. Adipocytes also express CB1 receptors, which are involved in energy metabolism[23,41]. Pharmacological CB1 receptor blockade increased adiponectin messenger RNA expression in adipose cells and tissue, respectively[23,50]. These results suggest that CB1 receptors on adipocytes normally suppress adiponectin activity. In addition, CB1-dependent mechanisms increase lipoprotein lipase activity enhancing the storage of more fat[41,51,52]. These results, together with the finding that other components of the ECS are found in fat tissue[53], demonstrate that endocannabinoid signaling can be an important target to modulate fat metabolism. Indeed, analysis of human adipose tissue of obese patients revealed a decrease in the activity of the main AEA-degrading enzyme fatty acid amide hydrolase, increased endocannabinoid levels and decreased expression of CB1 receptors[54]. Moreover, CB1 receptor activation results in adipogenesis and lipogenesis[41], which leads to an impaired mitochondrial function in diet-induced obesity[55]. Overall, the specific targeting of adipocyte CB1 receptors, which have been recently shown to play a key role in the crosstalk across adipocytes, immune cells and the sympathetic nervous system[23], represents an interesting therapeutic approach in order to treat obesity and metabolic syndrome.

Hepatic endocannabinoid signaling. The deletion of CB1 receptors specifically from mouse hepatocytes has brought important findings supporting the role of endocannabinoid signaling in the liver[56]. High-fat diet exposure of animals lacking hepatic CB1 receptors does not cause dyslipidemia, insulin/leptin resistance, hepatic steatosis, which are key metabolic consequences associated with the diet-induced obesity[57,58] (but see Ref. 59). In line with the general increase of endocannabinoid signaling under obesogenic conditions, high fat diet- or alcohol-induced hepatic steatosis is linked to an upregulation of liver CB1 receptors and the modulation of other components of the ECS[58]. Interestingly, studies on human patients with hepatic steatosis have also detected increases in endocannabinoid levels[60] and hepatic CB1R expression[61]. Moreover, hepatic CB1 receptors contribute to control bile acid metabolism whereas bile acids can control the levels of certain endocannabinoids[62]. However, the link between endocannabinoid signaling and bile acid composition and its impact on body weight control is still unknown.

Endocannabinoid signaling in other peripheral sites. All key players of the ECS are present in other peripheral tissues such as the gastrointestinal (GI) tract[24], where they regulate motility, acid and fluid release, inflammation, vasodilation, but also food intake[63]. In addition, endocannabinoids in the GI tract affect the secretion of hormones critically involved in whole body energy metabolism, such as ghrelin, CCK and GLP-1, thereby exerting a crucial role in inter-organ endocrine communication processes[63-65]. Neuronal bidirectional communication between the periphery and the brain is also under the control of endocannabinoid signaling, as strong evidence indicates that both the sympathetic and parasympathetic systems participate in its impact on metabolism and behavior[63,66-68]. The ECS is also known for its contribution to blood glucose control, mostly by the direct modulation of endocrine cell types of the pancreatic Langerhans islets[27], although the exact mechanisms are not known yet. Finally, peripheral endocannabinoid signaling is also present in skeletal muscle, where its activation decreases glucose uptake and oxidative metabolism[25].

3. Endocannabinoids and mitochondria.

History is an important factor to approach the study of the ECS. The search for the biological mechanisms underlying the effects of the *Cannabis sativa* plant became very strong in the late 1960s and in the 1970s, as depicted by the relatively rapid raise of publications during this period (Figure 1). However, this wave of interest was slowly decreasing during the 1980s, likely because the lack of a precise mechanism of action of cannabinoids made the research less attractive. By early 1990s, however, the discovery of cannabinoid receptors and endocannabinoids fueled again a rapidly growing and still ongoing raise of enthusiasm (Figure 1). Interestingly, a series of "pre-receptor" papers during the 1970s showed that plant-derived cannabinoids can alter mitochondrial activity by affecting, for instance, NADH oxidase activity[69], ATP synthesis[70] and oxygen consumption[71]. With the later discovery of cannabinoid receptors and their description as GPCRs, however, these intracellular effects were revised and finally considered as artifacts or unspecific drug effects. Indeed, lipid cannabinoids were shown to alter lipid membrane composition (including mitochondrial ones)[72], thereby possibly altering the

activity of organelles in an unspecific manner. As GPCRs have been always known to be "plasma membrane" proteins, the possibility that some of the cannabinoid effects on mitochondrial activity might be specifically and directly receptor-mediated was not taken into consideration. To the best of our knowledge, the first functional and anatomical evidence of a receptor-mediated regulation of mitochondrial activity by cannabinoids came in the years 2005-2010, when a series of studies showed the impact of cannabinoids on mitochondrial activity in human sperms[73-75], where the presence of CB1 receptors on mitochondrial membranes was first observed. The specificity of these extremely interesting human data could obviously not be confirmed by the use of samples genetically lacking the CB1 receptor. This was done in 2012, when the comparison and rigorous quantification of immunogold electron microscopy staining in the brain of wild-type and CB1-KO mice allowed establishing the specific presence of mitochondria-associated CB1 receptors (mtCB1)[76]. Likewise, the effects of cannabinoids on oxygen consumption in brain-isolated mitochondria was shown to be present in wild-type, but not in CB1-KO mice[76]. These data did not exclude that, depending on doses and conditions, cannabinoids might also unspecifically alter mitochondrial functions by modifying membrane lipid composition, but they strongly promoted the idea that GPCRs can be active intracellularly and regulate the functions of organelles in a specific way[77-80]. Importantly, Benard et al also showed that endocannabinoids are present in brain mitochondria and their levels are strikingly inversely proportional to the respiration activity of the organelles[76], indicating that the CB1-dependent modification of mitochondrial functions is not only a pharmacological effect of exogenous cannabinoids, but it represents a physiological feature of the ECS. The functional presence of mtCB1 has been so far shown in different tissues and cell types[25,73,76,81-84]. For instance, mtCB1 has been associated to the regulation of motility and other functions in sperms[73-75,85], muscle mtCB1 can regulate oxygen consumption[25], and the presence of mtCB1 in progesterone-producing ovarian cells, recently suggested its implication in peripheral hormonal regulation[82]. However, the brain is so far the organ where most details have been obtained concerning the functions and the mechanisms of action of mtCB1 receptors. Interestingly in the context of the present article, the discovery of mtCB1 added a new element to the chain linking (endo)cannabinoid signaling to metabolic processes of the cells. Thus, not only

endocannabinoids can regulate body energy metabolism by typical GPCR-like signaling at cellular plasma membranes, but they can use the modulation of cellular energy transformation as a signal pathway to control organ functions and, in the brain, to dictate behavioral choices. So far, amongst other behavioral processes, mtCB1 activity has been involved in the regulation of food intake[81,86], hippocampal memory[84], social interactions[83], nociception and motor control[86].

The brain is the most complex organ of the body and, accordingly, its bioenergetic requirements are very high. Many brain cells contribute to bioenergetic roles, but glial cells, and in particular astrocytes, are major effectors of central metabolic functions in mammals. In the next section, we will see how astroglial mtCB1 receptors can regulate behavior by reprogramming brain metabolic activity.

4. Endocannabinoids and astrocytes.

Neurons and astrocytes are metabolically different. For their bioenergetic needs, astrocytes mainly rely on glycolysis[87-90], whereas neurons use principally the mitochondrial oxidative phosphorylation (OXPHOS) pathway[91,92]. Given their glycolytic phenotype, astrocytes are net producers of lactate, a metabolite now considered an energy substrate for neighboring neurons[93,94]. This astrocyte-neuron intercellular metabolic coupling through lactate actually represents a key element sustaining brain functions[95] such as long-term memory formation[96] or depressive-like behaviors[97].

OXPHOS, besides being a major site for ATP generation, actively produces reactive oxygen species (ROS) with physiological signaling properties[98]. Mitochondrial complex I (CI) is an important site for ROS production under physiological conditions[99] that is amenable to regulation by assembling with complex III (CIII)[100]. In astrocytes, CI is partially disassembled from CIII, determining low OXPHOS energy efficiency, but high ROS generation under physiological conditions[100]. Indeed, downmodulating mitochondrial ROS abundance in astrocytes by a genetic approach causes profound metabolic changes, negatively impacting on neighboring neuronal integrity and behavior in mice[101].

Interestingly, recent work proposed a possible connection between mtCB1-mediated inhibition of CI activity in the brain[76,84] and CI production of ROS in astrocytes[83]. For ROS formation, CI requires an intact N-module, i.e., the functional domain of the complex that is responsible for electron acceptance from NADH(H+)[102,103]. Activation of mtCB1 in astrocytes causes the loss of CI N-module stability, without affecting those of the Q- and the P-modules[83]. Earlier studies revealed that the PKA-dependent phosphorylation of the CI N-module subunit NDUFS4 is required to sustain CI activity[104]. Interestingly, activation of mtCB1 causes NDUFS4 dephosphorylation that wholly accounts for the loss of the CI N-module stability and the impairment of mitochondrial ROS formation in astrocytes[83]. Notably, mitochondrial ROS stabilizes hypoxia-inducible factor-1a (HIF1a)[105], a transcription factor that sustains the expression of glycolytic genes[106]. In line with this, mtCB1-induced decrease of ROS causes HIF1a destabilization and debilitates glycolysis in astrocytes, leading to a reduced release of astroglial lactate, eventually causing neuronal bioenergetic dysfunction[83]. Interestingly, this cascade results in reduced mouse social interactions, which can be reverted by exogenous lactate[83]. Thus, the mtCB1-dependent control of cellular metabolic pathways is translated into key behavioral functions.

4. Conclusions and future perspectives

Metabolism can be defined as "the chemical processes that occur within a living organism in order to maintain life". Endocannabinoids are surely important metabolic molecules both at cellular and whole-body levels. As such, they control energy metabolism, even beyond the immediate needs, to "be ready" for future scarcity of energy sources and, thereby to maintain life through "exostatic" mechanisms. This is obtained by a myriad of alternative, overlapping or distinct cellular mechanisms that rely on different signaling couplings and/or subcellular locations of the cannabinoid receptors. The continuous identification of new mechanisms and/or new locations will tell us how many cellular processes can be impacted by the endocannabinoid signaling. The discovery that endocannabinoids can alter cellular mitochondrial functions represents a key step towards a full understanding of their metabolic impact. Moreover, the cumulating evidence that other GPCRs are also functionally present at

mitochondrial membranes suggests that the control of bioenergetic processes can be a generalized means of receptor-dependent regulation of cell metabolism and functions. In this context, the fact that typical signaling receptors directly "use" metabolic processes to signal, suggests that the classical separation between metabolism and signaling is possibly less clear cut than previously thought. So far, mtCB1 receptors have been mainly studied as the pharmacological targets of exogenous cannabinoid drugs. Interestingly, the data seem to indicate that whereas certain cannabinoid effects (e.g. analgesia) do not require mtCB1 receptors, undesired side-effects of these drugs such as decreased social behavior, impairment of memory or alteration of motor responses are mediated by direct mitochondrial alterations. All these observations are necessarily partial, but they suggest that also different physiological functions of endocannabinoids might require or not mitochondrial and metabolic signaling. In the brain, astrocytes are key metabolic cells, whose activity is largely dedicated to the maintenance of energy levels in the organ. The fact that astroglial metabolism is under endocannabinoid control is a further indication of the key metabolic functions of these molecules. However, astrocytes are not only metabolic cells and strongly participate in the signaling necessary for synaptic activity. The recent results indicating that endocannabinoids can control synaptic plasticity by regulating mitochondrial calcium signaling[107] is another clear indication that metabolic processes can be directly used as signaling in the brain. In addition, the control of body metabolism is clearly the result of a constant interaction between the periphery and the brain. Endocannabinoid signaling, through its almost ubiquitous presence and its ability to modulate key metabolic, hormonal and neuronal processes, is perfectly placed to control this bidirectional interaction. Future studies will for sure reveal new aspects and implications of this pivot role of endocannabinoids.

This short article tried to provide a rapid overview of some of the myriad pieces of evidence that support the key role of endocannabinoids in the control of body and cellular metabolism and of how this control translates into regulation of very different processes, from biochemistry to behavior. The exciting avenues opened by old and recent discoveries on endocannabinoid signaling will lead us to a better understanding of metabolic regulation and to the design of novel approaches for the treatment of

metabolic disorders. Moreover, accumulating evidence indicates that many diseases, whose metabolic component has been so far overlooked, actually involve alterations of cellular metabolism at different levels. Therefore, the raising role of endocannabinoids in the control of this function will extend our understanding of the therapeutic properties of cannabinoid-related drugs.

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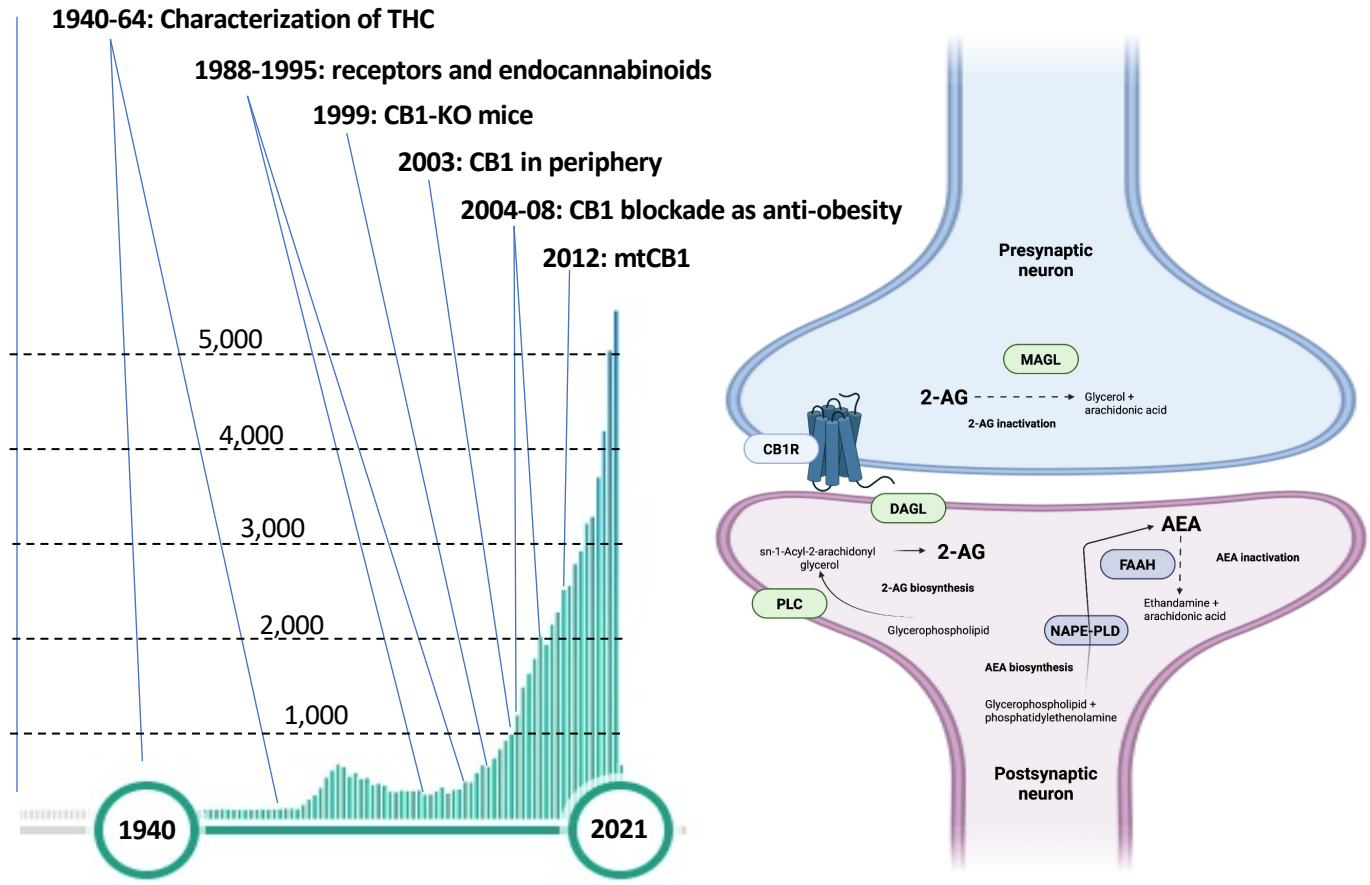
Figure Legends

Figure 1. Timeline of (endo)cannabinoid research. On the left, Timeline of recorded publications (green bars) using the keywords "Cannabis OR Cannabinoid OR Endocannabinoid" (source PubMed, https://pubmed.ncbi.nlm.nih.gov/?term=cannabinoid+or+endocannabinoid+or+cannabis). Time is selected after 1940 to improve clarity (No more than 1-2 publications per year from 1840s until 1940s). First publications on cannabis appear in the middle of 1800, when the plant is imported from East. The chemical composition of the plant is characterized between 1940 and 1964. Notice the peak of publications on the subject in the following period. The number of publications then decreases, likely reflecting the difficulty in finding a clear mechanism of action of cannabinoids. Publication rate starts again to grow exponentially after the characterization of receptors and endocannabinoids around early 1990s. The number of publications per year did not stop growing since. In 2021, 5,302 papers appeared on the subject. Adding the keyword "metabolism" reduces the absolute numbers (2,923 papers in 2021) but maintains the same trend overtime (not shown). On the right, schematic and simplified representation of the main processes involved in the synthesis and degradation of the two main endocannabinoids, 2-AG and AEA.

Figure 2. Target locations and metabolic activities of endocannabinoids. Endocannabinoids and their receptors (here CB1 receptors) are highly conserved between mice and humans. Most observations have been made in rodents but are also supported by human data. Amongst other functions, endocannabinoids control brain functions to increase food intake (particularly palatable and energy-rich) and regulate bioenergetic functions such as mitochondria respiration and astroglial glucose metabolism. In addition, endocannabinoids increase lipogenesis in the liver, increase insulin secretion in the pancreas, increase nutrients absorption in the intestinal tract, decrease glucose use in the muscle and favor the accumulation of fat in the adipose system.

Figure 3. Diverse CB1R signalling cascades and their functions. A representative and simplified description of the CB1 receptor signalling depending on their location is shown: 1) CB1 receptors are (mostly) integrated in the membranes of presynaptic neurons where are

652 coupled to Gi/o proteins that control several signalling cascades including the PI3K-AKT, the
653 MAPK and the PKA cascades. This neuronal CB1 receptors are involved in the control of
654 neurotransmission (NT) and cell survival. 2) Astroglial CB1 receptors are mainly coupled to
655 Gq protein. Their activacion increases calcium influx and regulates gliotransmission, among
656 other processes. As represented in the Figure, other G proteins have been linked with CB1
657 receptors being involved in CB1 signalling. 3) CB1 receptors associated to mitochondrial
658 membranes are involved in the regulation of mitochondrial respiration through a specific
659 signalling cascade, which impact neuronal functions and astroglial glycolisis and lactate
660 release. In adddition,astroglial mtCB1 receptors are involved in the calcium transfer
661 between ER and mitochondrial, which impacts lateral synaptic potentiation.
662



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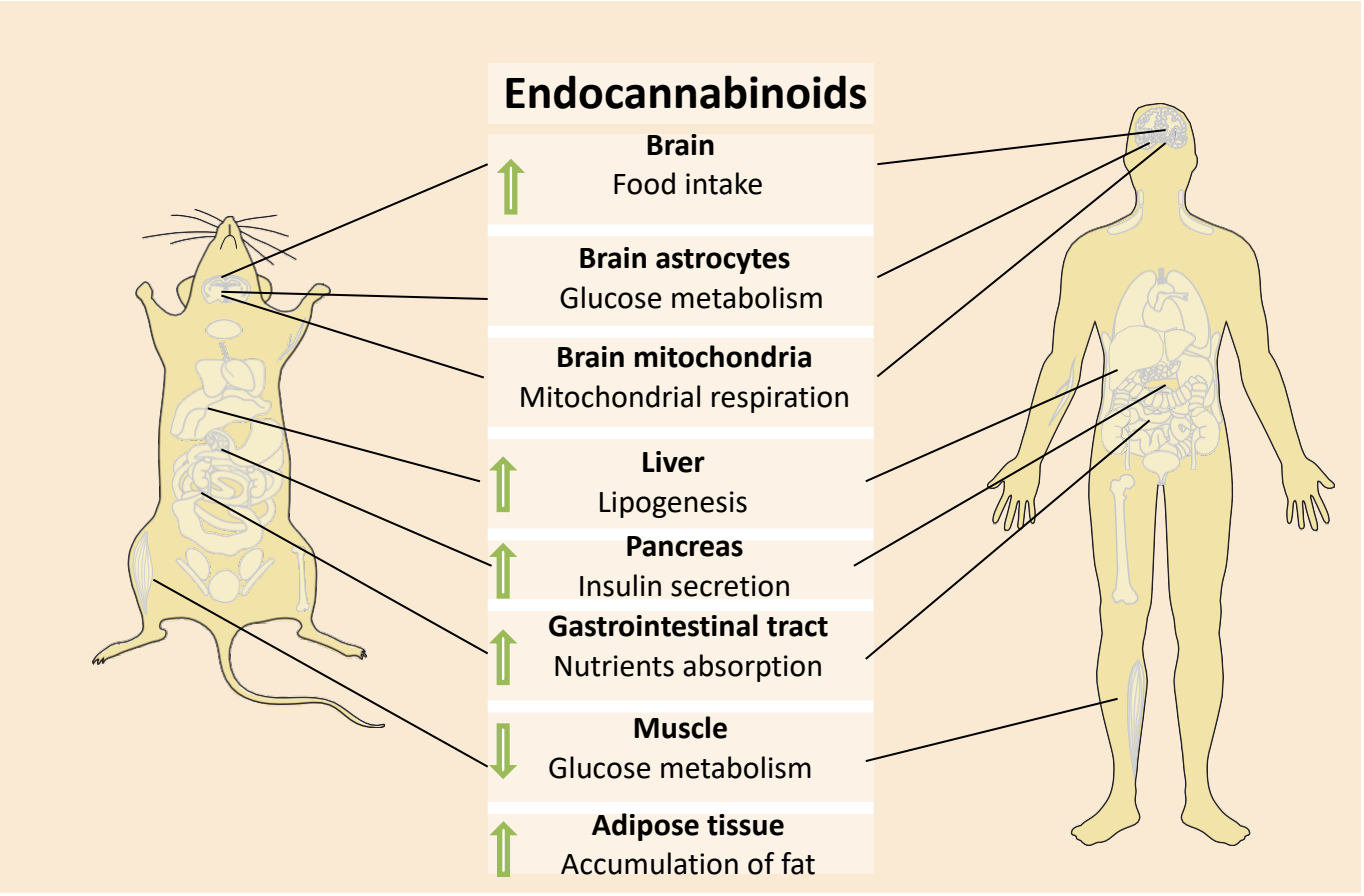

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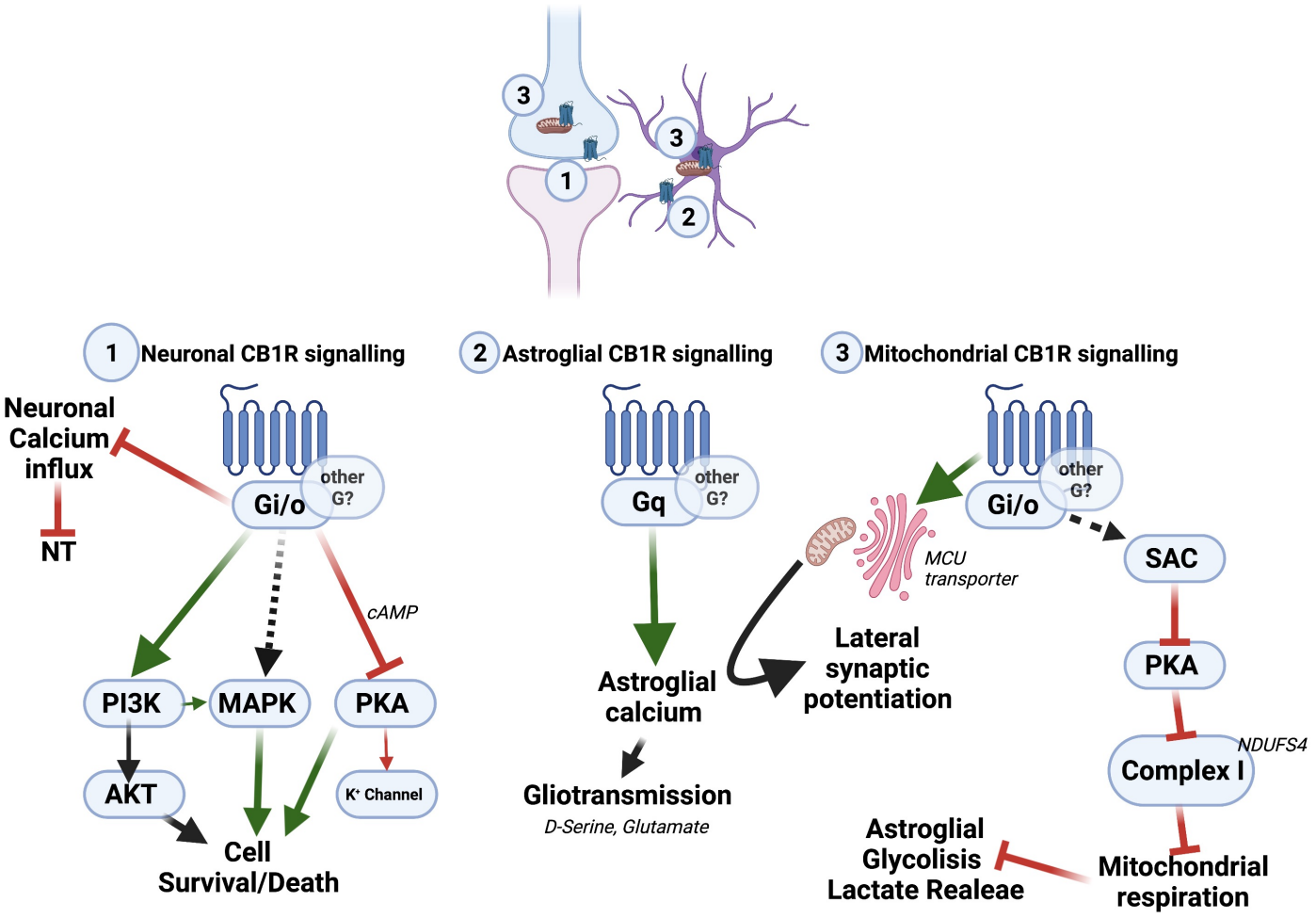

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