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## 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.

## 25 **1. Introduction**

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

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

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

55 Receptors (GPCRs) and they were first discovered as the cellular targets of exogenous  
56 plant-derived (THC) or synthetic cannabinoids<sup>6,7</sup>. This discovery implied the existence of  
57 endogenous ligands, which were soon identified as lipid derivatives of arachidonic acid  
58 and called endo-cannabinoids<sup>8,9</sup>. The most studied endocannabinoids are 2-  
59 arachidonoyl-glycerol (2AG) and anandamide (AEA), but new concepts have arisen that  
60 should be taken into consideration when dealing with endocannabinoid signaling: (i)  
61 2AG and AEA could bind to other targets than cannabinoid receptors<sup>14</sup>, implying that  
62 their tissue level changes do not always reflect an activation of the ECS in strict sense;  
63 (ii) peptide endogenous ligands (PepCans) can bind and modulate the activity of  
64 cannabinoid receptors and were proposed to belong to the endocannabinoid family<sup>16,17</sup>;  
65 (iii) the synthetic and catabolic pathways of lipid endocannabinoids are redundant and  
66 involve also other lipids, such as prostaglandins and others<sup>18</sup>; (iv) non-arachidonic acid-  
67 derived lipids were identified as endogenous allosteric regulators of cannabinoid  
68 receptor activity, such as the anti-inflammatory Lipoxin A4 (Ref. 19) or the steroid  
69 precursor Pregnenolone<sup>20</sup>, making these molecules members *de facto* of the ECS.

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

71  
72 Few attempts have been made to obtain a unified vision of the large spectrum of  
73 functions controlled by endocannabinoid signaling in animal physiology. One of the  
74 most convincing ideas in our opinion is that endogenous CB1 receptor signaling might  
75 represent a powerful system to adjust body metabolism towards the accumulation of  
76 energy reserves. As such, endocannabinoid signaling would exert a prototypical  
77 exostatic function (accumulation of reserves for future potential needs), as opposed to  
78 endostatic ones, through which the body responds to present needs (see Ref. 21 for an  
79 exhaustive discussion). Thus, amongst other functions, endocannabinoids can rewire  
80 behavior towards more food intake (particularly palatable and energy-rich)<sup>22</sup>, favor the  
81 accumulation of fat in the adipose system<sup>23</sup>, increase nutrients absorption in the  
82 intestinal tract<sup>24</sup>, decrease glucose use in the muscles<sup>25</sup>, increase lipogenesis in the  
83 liver<sup>26</sup> and increase insulin secretion in the pancreas<sup>27</sup> (Figure 2). In line with this, other  
84 roles of endocannabinoid signaling such as in the reduction of passive fear responses<sup>28</sup>,  
85 of pain<sup>29</sup>, of anxiety<sup>30</sup>, or the gating of motivation for physical exercise<sup>31-33</sup> would help  
86 individuals to be resilient against adversities and to physically engage in the search and

87 consumption of food<sup>21</sup>. Similarly, endocannabinoids seem to enhance sensory  
88 perception, like vision, olfaction or taste<sup>34,35</sup>, all conditions that would favor the  
89 detection of nutrients in the environment<sup>21</sup>. Thus, endocannabinoids play a key role in  
90 the control of body energy metabolism.

91 The link between endocannabinoid signaling and energy metabolism is based on an  
92 overwhelming amount of experimental and clinical data. In the second half of the years  
93 2000, the CB1 receptor blocker rimonabant was commercialized as a therapeutic aid  
94 against obesity and associated metabolic disorders<sup>36</sup>. Before being withdrawn in 2008  
95 because of undesired side effects linked to anxiety and depression<sup>37</sup>, rimonabant  
96 showed its efficacy in reducing not only body weight, but also many other obesity-  
97 associated metabolic dysfunctions<sup>36,38,39</sup>. These aspects have been listed and discussed  
98 by several reviews over the years<sup>3,40,41</sup>. In this section, we will just shortly highlight how  
99 endocannabinoid signaling through CB1 receptors can impact central and peripheral  
100 mechanisms involved in energy metabolism. For space reasons, we will focus on CB1  
101 receptor signaling, but it should be considered that the other main cannabinoid  
102 receptors (*i.e.*, CB2 receptors) have also been involved in whole-body metabolism<sup>42,43</sup>.

103  
104 *Neuronal endocannabinoid signaling.* CB1 receptors in the brain, especially those  
105 located in the hypothalamic areas, play a key role in controlling appetite and  
106 modulating certain neuropeptides to favor energy accumulation and storage<sup>3,44</sup>. In  
107 other brain regions involved in reward (*i.e.*, ventral tegmental area or nucleus  
108 accumbens), the endocannabinoid signaling has also been linked to the control of  
109 hedonic feeding behavior<sup>45,46</sup>. As happen with other brain functions<sup>47</sup>, depending on the  
110 brain regions where CB1 receptors are located, the control over food intake or energy  
111 balance can be very different. Indeed, this control also depends on whether CB1  
112 receptors are located on specific cell types<sup>22,48</sup>. Thus, specific pools of CB1 receptors  
113 present in particular hypothalamic cells, such as the POMC neurons, or in the  
114 sympathetic system could modulate food intake<sup>3,44,48</sup>. Accordingly, different doses of  
115 (endo)cannabinoids could preferentially act on glutamatergic or GABAergic CB1  
116 receptors to respectively increase or decrease food intake in mice<sup>22</sup>. Similar biphasic  
117 effects of cannabis on food consumption were also observed in humans<sup>49</sup>.

118

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

136

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

151

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

166

### 167 **3. Endocannabinoids and mitochondria.**

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

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



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

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

#### 225 **4. Endocannabinoids and astrocytes.**

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

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

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

259

#### 260 **4. Conclusions and future perspectives**

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

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

298

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

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

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311

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619

620 **Figure Legends**

621

622 **Figure 1. Timeline of (endo)cannabinoid research.** On the left, Timeline of recorded  
623 publications (green bars) using the keywords “Cannabis OR Cannabinoid OR  
624 Endocannabinoid” (source PubMed,  
625 <https://pubmed.ncbi.nlm.nih.gov/?term=cannabinoid+or+endocannabinoid+or+cannabis>).

626 Time is selected after 1940 to improve clarity (No more than 1-2 publications per year from  
627 1840s until 1940s). First publications on cannabis appear in the middle of 1800, when the  
628 plant is imported from East. The chemical composition of the plant is characterized between  
629 1940 and 1964. Notice the peak of publications on the subject in the following period. The  
630 number of publications then decreases, likely reflecting the difficulty in finding a clear  
631 mechanism of action of cannabinoids. Publication rate starts again to grow exponentially  
632 after the characterization of receptors and endocannabinoids around early 1990s. The  
633 number of publications per year did not stop growing since. In 2021, 5,302 papers appeared  
634 on the subject. Adding the keyword “metabolism” reduces the absolute numbers (2,923  
635 papers in 2021) but maintains the same trend overtime (not shown). On the right, schematic  
636 and simplified representation of the main processes involved in the synthesis and  
637 degradation of the two main endocannabinoids, 2-AG and AEA.

638

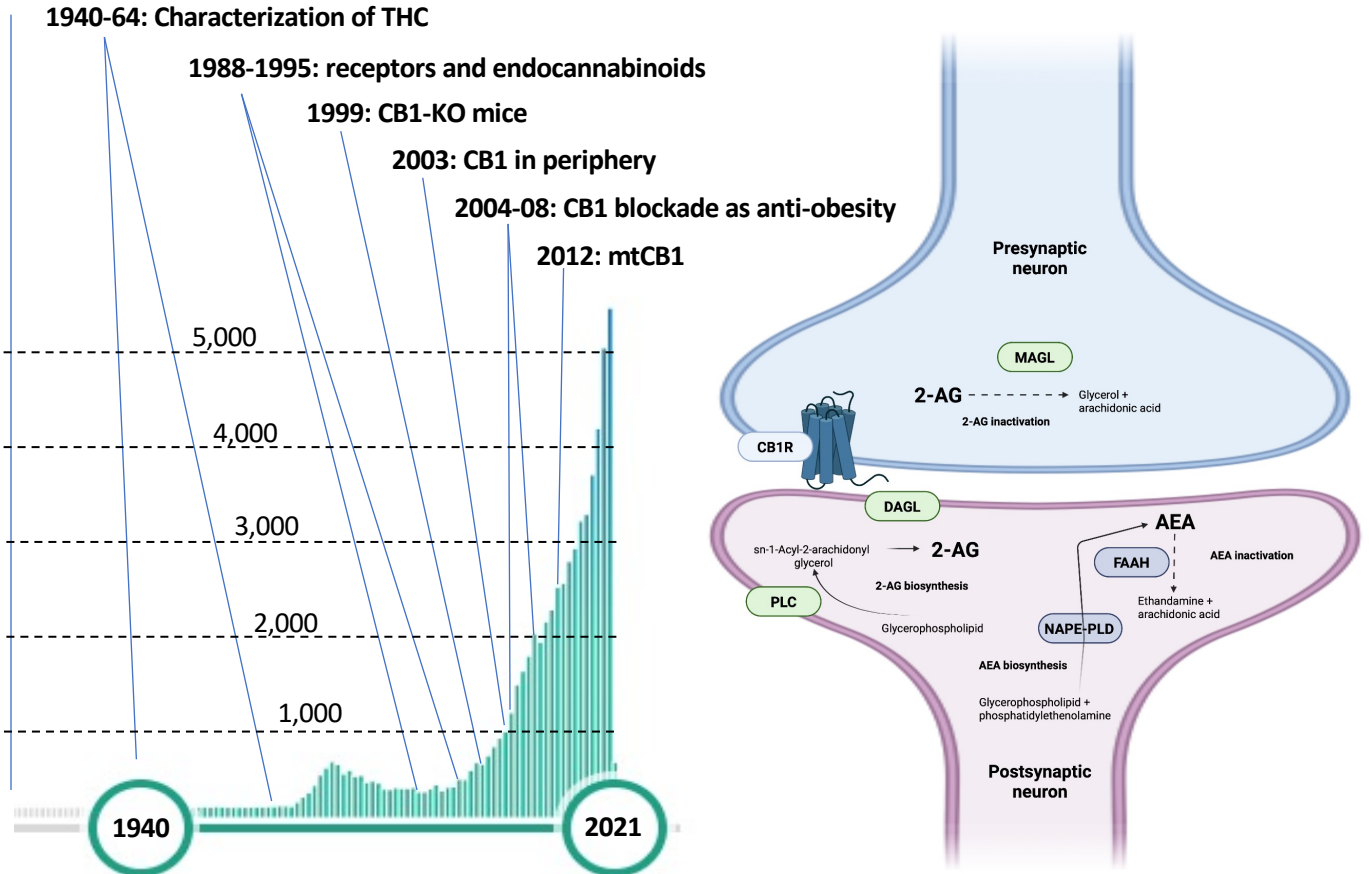
639 **Figure 2. Target locations and metabolic activities of endocannabinoids.** Endocannabinoids  
640 and their receptors (here CB1 receptors) are highly conserved between mice and humans.  
641 Most observations have been made in rodents but are also supported by human data.  
642 Amongst other functions,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

648

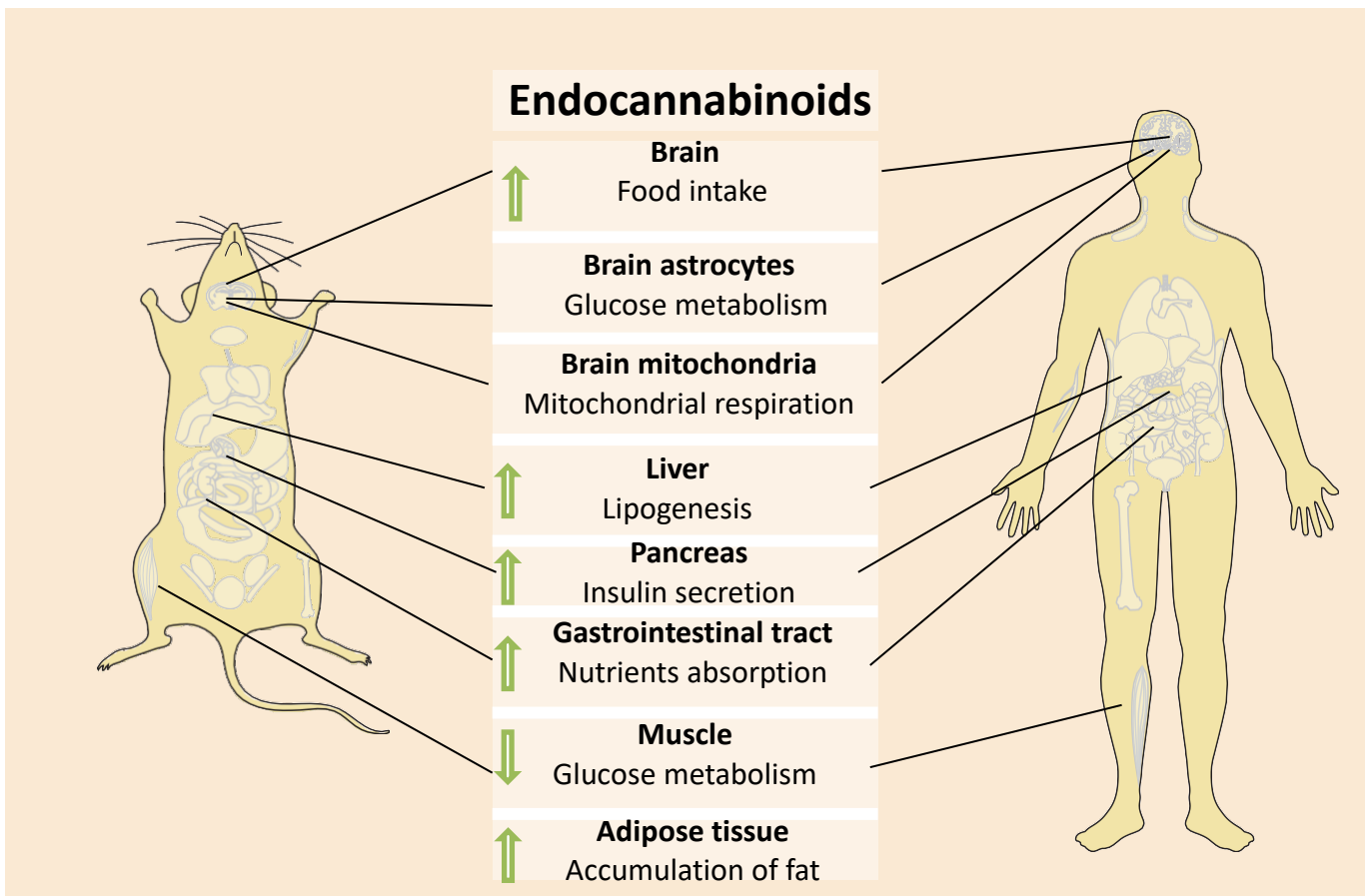
649 **Figure 3. Diverse CB1R signalling cascades and their functions.** A representative and  
650 simplified description of the CB1 receptor signalling depending on their location is shown: 1)  
651 CB1 receptors are (mostly) integrated in the membranes of presynaptic neurons where are

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 activation 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 glycolysis and lactate  
660 release. In addition, astroglial mtCB1 receptors are involved in the calcium transfer  
661 between ER and mitochondrial, which impacts lateral synaptic potentiation.  
662

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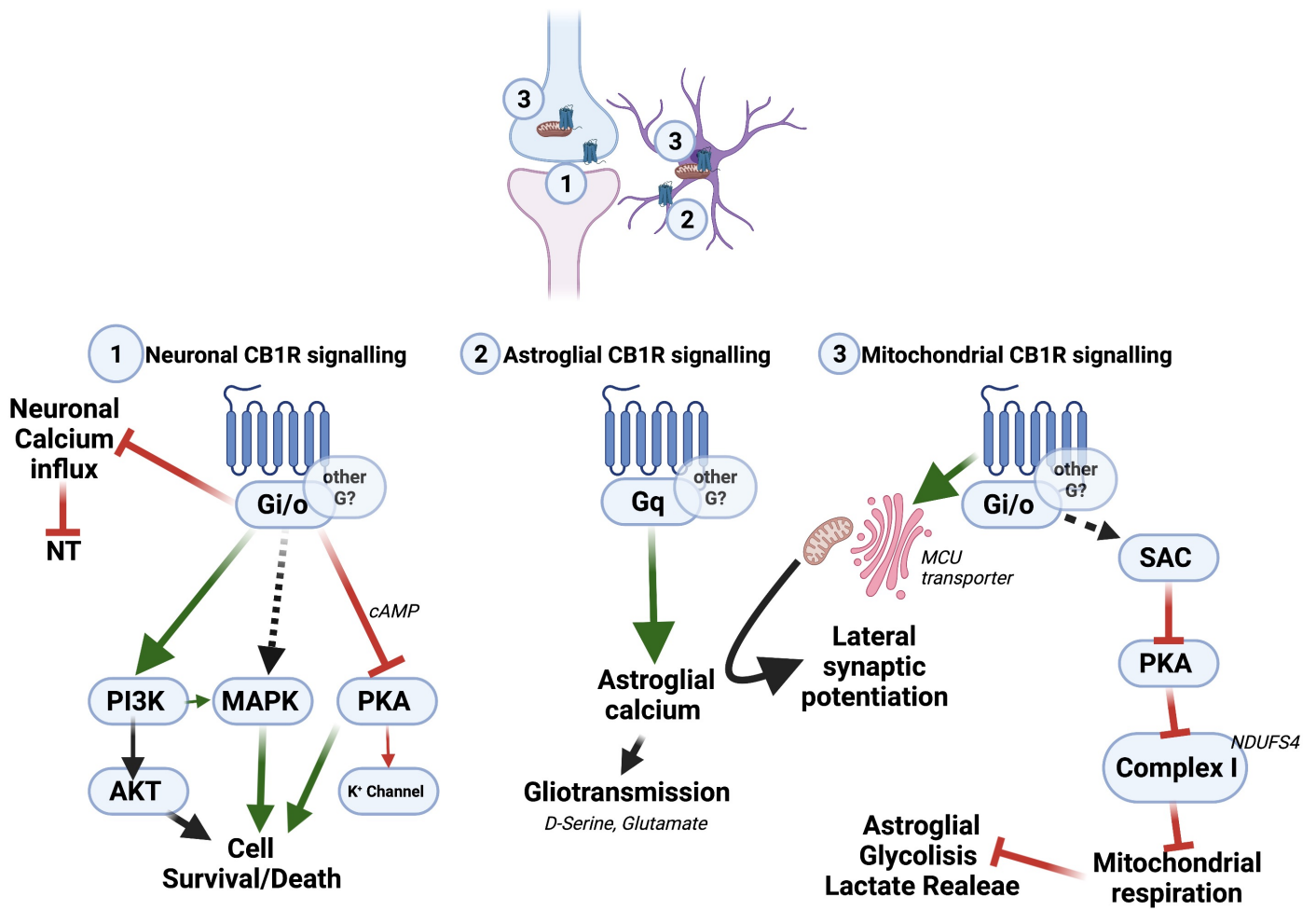


**Figure 1. Timeline of (endo)cannabinoid research.** On the left, Timeline of recorded publications (green bars) using the keywords “Cannabis OR Cannabinoid OR Endocannabinoid” (source PubMed, <https://pubmed.ncbi.nlm.nih.gov/?term=cannabinoid+or+endocannabinoid+or+cannabis>). Time is selected after 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 in order 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 coupled to Gi/o proteins that control several signalling cascades including the PI3K-AKT, the MAPK and the PKA cascades. This neuronal CB1 receptors are involved in the control of neurotransmission (NT) and cell survival. 2) Astroglial CB1 receptors are mainly coupled to Gq protein. Their activation increases calcium influx and regulates gliotransmission, among other processes. As represented in the Figure, other G proteins have been linked with CB1 receptors being involved in CB1 signalling. 3) CB1 receptors associated to mitochondrial membranes are involved in the regulation of mitochondrial respiration through a specific signalling cascade, which impact neuronal functions and astroglial glycolysis and lactate release. In addition, astroglial mtCB1 receptors are involved in the calcium transfer between ER and mitochondrial, which impacts lateral synaptic potentiation.