

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Pharmacological Actions and Potential Therapeutic Use of Cannabinoids in Duchenne's Muscular Dystrophy

Fabio Arturo Iannotti

Abstract

The scientific community uses the term endocannabinoid system (ECS) to refer to a large group of molecules that in our body control the production and function of the two major cannabinoid lipid mediators, namely, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Following their discovery, an impressive number of studies have shown that both AEA and 2-AG play a key role in a large plethora of functions in living organisms. Consequently, functional impairment or dysregulation of AEA and 2-AG activity leads to a variety of disorders affecting the nervous system as well as peripheral organs and tissues. For this reason, cannabinoids and/or cannabinoid synthetic drugs currently represent an important area of research for their potential therapeutic use to treat many human diseases having or not a genetic component. Despite these evidences, the role of the endocannabinoid system and hence potential changes in its activity in inherited muscular dystrophies remains largely unknown. Only recently, the role of endocannabinoid CB1 receptors was identified in Duchenne's muscular dystrophy (DMD). In this chapter, I summarize the chemical properties and functional role of the endocannabinoids as well as plant-derived cannabinoids during skeletal muscle formation and repair under physiological conditions as well as DMD.

Keywords: endocannabinoid system (ECS), endocannabinoids (ECs), cannabidiol (CBD), cannabidivarin (CBDV), cannabinoid receptor of type 1 (CB1), Duchenne's muscular dystrophy (DMD), transient receptor potential cation channels (TRP channels), anandamide (AEA), 2-arachidonoylglycerol (2-AG)

1. Introduction

1.1 Description of the endocannabinoid system (ECS)

The term endocannabinoid system (ECS) was originally coined in the 1990s after the discovery of brain receptors, responsive to Δ^9 -tetrahydrocannabinol-THC (the primary psychoactive substance of *Cannabis sativa*) and the class of their endogenous ligands identified immediately thereafter [1]. Since these discoveries, the number of molecules functionally associated with the activity of the endocannabinoid system has grown exponentially.

Nowadays, the ECS is considered to be a very complex network of connected signaling molecules, and key components of this system include (a) the two most potent endogenous agonists of cannabinoid receptors, anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG), also named endocannabinoids; (b) endocannabinoid-related molecules including N-oleoylethanolamine (OEA) and N-palmitoylethanolamide (PEA); (c) the enzymes regulating the endocannabinoid biosynthesis (NAPE-PLD, ABDH4, GDE1, PTPN22 for AEA, and DAGL α and DAGL β for 2-AG) and degradation (FAAH for AEA and MAGL, ABDH6, ABDH12, and FAAH for 2-AG); (d) the two endocannabinoids responsive to G-protein-coupled receptors known as cannabinoid receptor of type 1 (CB1) and type 2 (CB2); and (e) the cation permeant transient receptor potential vanilloid type-1 (TRPV1) [2–4]. Recently, AEA and 2-AG were also shown to have affinity for non-cannabinoid receptors including GABA-A, PPAR γ , adenosine A3, and GPR55 [5]. In this complex scenario, also other endogenous AEA and 2-AG analogues, including other N-acyl-ethanolamines (NAEs), monoacylglycerols, N-acyl amino acids, and N-acyl-dopamines/taurines/serotonins, were suggested to share, to some extent, either anabolic or catabolic pathways, or both, with endocannabinoids (**Figure 1**) [6].

As also mentioned previously, the endocannabinoid system (ECS) is critically involved in regulating a variety of metabolic and cognitive processes. An overactive endocannabinoid/CB1R system has been associated with the development of obesity, insulin resistance, and dyslipidemia [7–10] as well as during the progression of neurological disorders such as Alzheimer's disease, multiple sclerosis, amyotrophic

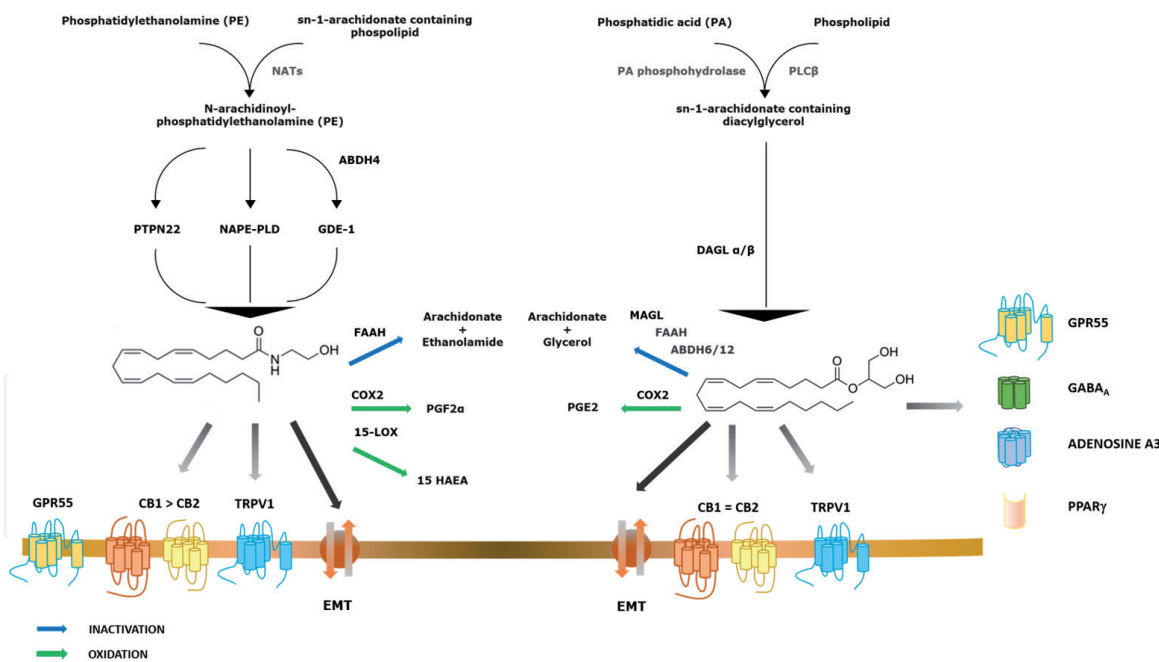


Figure 1.

Synthesis, inactivation, and mechanism of action of the two endocannabinoids anandamide and 2-AG. Thick black arrows indicate the biochemical reactions that starting from the precursor membranes lead to the synthesis of the two endocannabinoids anandamide and 2-AG. ABDH4, α β -hydrolase 4; ABDH6, α β -hydrolase 6; ABDH12, α β -hydrolase 12; CB1 and CB2, cannabinoid receptor of types 1 and 2; COX2, cyclooxygenase 2; DAG, diacylglycerol; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; GDE1, glycerophosphodiester phosphodiesterase 1; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acyl-phosphatidylethanolamine-selective phosphodiesterase; NATs, N-acyltransferases; PA, phosphatidic acid; PLC β , phospholipase C β ; PLD, phospholipase D; 15-LOX, 15-lipoxygenase; PTPN22, protein tyrosine phosphatase, non-receptor type 22; PGF2 α , prostaglandin F2 α ; 15 HAEA, 15(S)-HETE ethanolamide; PGE2, prostaglandin E2; TRPV1, transient receptor potential, vanilloid subtype 1 receptor (this figure was copied directly from Arturo, Iannotti Fabio, and Fabiana, Piscitelli (Nov. 2018) Endocannabinoidome. In: eLS. John Wiley & Sons Ltd, Chichester). <http://www.els.net> [doi: 10.1002/9780470015902.a0028301].

lateral sclerosis, Parkinson's disease, and Huntington's chorea [4, 11]. Nevertheless, the potential role of the ECS in skeletal muscle disorders remains largely unknown.

2. What is known about the role of endocannabinoid systems in skeletal muscle

2.1 The role of the endocannabinoid system on glucose metabolism and insulin sensitivity in skeletal muscles

The first evidence demonstrating that CB1 receptors are functionally expressed in skeletal muscle tissues came out in 2005, when Liu and colleagues reported the effects of SR141716 (commonly known as rimonabant), one of the most used selective CB1 antagonists/inverse agonists [12], on energy expenditure and glucose uptake in isolated soleus muscle of obese *Lep(ob)/Lep(ob)* mice. In particular, the authors found that 5 days after daily treatment, SR141716 resulted in a significant reduction of daily food intake and body weight. While after 7 days, SR141716 had also positive effects on basal oxygen consumption and glucose uptake [13].

Few years later, Cavuoto and colleagues in two parallel studies demonstrated first that not only CB1 but also CB2, TRPV1, and FAAH are expressed in human and rodent skeletal muscle. Then, in human primary myotubes isolated from lean and obese donors, they found that the exposure to AEA or AM251 (another largely used selective CB1 antagonist), separately or in combination for 24 hours, induces significant changes in transcript levels of key genes regulating the metabolism such as AMP-activated protein kinase (AMPK) alpha 1 (alpha1) and alpha 2 (alpha2), pyruvate dehydrogenase kinase 4 (PDK4), and peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) [14, 15].

Thus, these pioneering studies have paved the way for other studies through which the role of the ECS in regulating energy balance at the skeletal muscle level was further strengthened. In this regard, Esposito and colleagues showed that in differentiated L6 myotubes, the pharmacological blockade by SR141716 or genetic silencing of CB1 small interfering RNA sequences increased 2-deoxy-glucose uptake (2-DG) in a time- and dose-dependent manner. The authors also demonstrated that, the activity of phosphatidylinositol-3-kinase (PI3K) that in turn has stimulatory effects phosphoinositide-dependent kinase-1, Akt/protein kinase B, and protein kinase C ζ , resulted increased by SR141716 [16]. Accordingly, Eckardt et al. found that in human skeletal muscle cells 24 hours of incubation with adipocyte-conditioned medium (CM) or anandamide (AEA) impaired insulin-stimulated Akt(Ser473) phosphorylation. By contrast, pretreatment with rimonabant or AM251 reduced the effect of CM by about one-half, while the effect of AEA was fully prevented. The reduction of insulin-stimulated glucose uptake by CM was completely prevented by rimonabant. In addition, AEA was found to transiently activate ERK1/ERK2 and p38 mitogen-activated protein kinase and impaired insulin-stimulated Akt (Ser473) phosphorylation, but had no effect on Akt (Thr308) and glycogen synthase kinase 3 alpha/beta phosphorylation. Surprisingly, after 24 hours of treatment, an enhanced IRS-1 (Ser307) phosphorylation induced by AEA was observed in human skeletal muscle cells [17]. Furthermore, activation or inhibition of CB1 receptor activity exerts a differential effect with regard to MAP kinase- and PKB-directed signaling [18]. In conclusion, all these studies provide robust evidence that the endocannabinoid "tone" (hence CB1 signaling) is dysregulated during the obesity where its overactivity generates detrimental consequences on insulin sensitivity and consequently glucose catabolism in skeletal muscle cells.

It is worth noticing, however, that there are some discrepancies in scientific literature on whether the stimulation or inhibition of CB1 receptors has positive or negative effects on glucose metabolism in skeletal muscles. These differences are likely due to the dose/concentration and incubation time of agonists and antagonists of CB1 or more simply to the experimental model used. For a more extensive overview on the results aforementioned, Heyman et al. have recently published an extensive literature review [19].

2.2 Changes in the ECS activity in response to physical exercise

In addition to the diet, the physical exercise also contributes to induce changes in ECS activity. In this regard, Sparling et al. were the first in 2003 to find a robust increase of AEA in plasma of young male volunteers subjected to a physical exercise of moderate intensity. This trend of increase, but much less prominent, was observed also for 2-AG [20]. Other studies have further demonstrated the close relationship between the intensity and type of physical exercise and ECS activity. In particular, while an exercise of moderate intensity was confirmed to increase the plasma levels of AEA in human volunteers, surprisingly no changes in plasma ECs were observed following a very-high- and very-low-intensity exercise [21–23]. Of note, the increased ECS activity induced by exercise was positively correlated with the beneficial antidepressant effects of exercise at central as well as peripheral levels including sense of well-being, anxiety reduction, postexercise calm, and reduced pain sensation [20, 23–26]. Interestingly, Hill et al. found that augmentation of exercise-induced increase of endocannabinoid tone suppresses stress-associated behaviors and promotes hippocampal cell proliferation, in a manner dependent on stimulation of CB1 stimulation [27, 28]. In addition, Heyman et al. provided evidence in humans that following acute exercise AEA and BDNF, a key neurotrophic factor regulating the brain development and cognitive functions [19], were positively correlated at the end of exercise and after the 15-min recovery.

As yet, the genetic deletion of CB receptors from the brain on VTA GABAergic neurons decreased wheel-running performance in mice [29]. However, quite surprisingly, when Gamelin et al. evaluated changes in the levels of AEA and 2-AG as well as of congener molecules in high-fat (HFD) diet subjected to 12 weeks of exercise training, they found that the high-fat diet paired with exercise training had no effect on AEA, 2-AG, and AEA congener levels nor in the hypothalamus or hippocampus. However, CB1 expression levels were significantly increased in the hippocampus in response to HFD, exercise, and the combination of both, strengthening evidence for EC signaling involvement in neuronal plasticity following diet and/or exercise [30]. A more recent study shows differences in the circulating levels of 2-AG and AEA between male and female mice or between lines of mice bred for high levels of voluntary exercise, when compared to their nonselected control lines [31]. An increased ECS activity was also recently found in skeletal muscles of mice subjected to muscle atrophy induced by mechanical unloading, most likely due to the disuse-induced muscle inflammation or the altered glycolytic flux [32].

2.3 Changes in the ECS during the skeletal muscle formation

In spite of the important role played by the ECS in regulating the insulin sensitivity and oxidation pathways in skeletal muscle cells, its potential involvement during skeletal muscle formation and regeneration is little known. In 2014, our research group has demonstrated for the first time that the 2-AG levels significantly declined after 3 days of murine C2C12 myoblasts exposure to differentiation media and remained reduced up to at least day 7 of the differentiation process. In contrast,

AEA was not significantly changed during the myotube formation. According to these results, we have also demonstrated that during myotube formation the expression profile of the entire class of genes involved in AEA synthesis and degradation was not significantly changed, whereas the expression of key genes regulating the 2-AG metabolism including *Dagl α* and *Magl* was significantly decreased and increased, respectively. Of note, potential changes in the endogenous levels of AEA and 2-AG were also explored during the skeletal muscle formation *in vivo*. In this case, we found that the levels of AEA were reduced in murine quadriceps muscle between the embryonic (E18) and early postnatal (P4) conditions, to then rebound at postnatal day 14 (P14). On the other hand, 2-AG levels remained constant between the embryonic and early postnatal phases but then decreased by about 50% at P14. In summary, there is evidence that during myogenesis, occurring both *in vitro* or *in vivo*, the endogenous levels of 2-AG decline with myotube formation and muscle growth, respectively. By contrast, the levels of AEA did not during the myotube formation *in vitro* but undergo oscillatory variations during muscle formation *in vivo* [33].

Furthermore, by the use of both pharmacological tools and techniques of gene silencing, we demonstrated that in murine and human myoblasts as well as human primary satellite cells, the stimulation of CB1 receptors with either exogenous or endogenous cannabinoids promotes myoblasts proliferation and inversely inhibits their differentiation in mature myotubes [33]. As yet, the stimulation of CB1 inhibits the activity of *K_v7* (or *KCNQ*) channels, a subclass of voltage-gated K^+ channels composed of five members (*K_v7.1–K_v7.5*) that once activated promote myogenesis [33–35].

It is worth noting that both proliferation and differentiation of muscle precursor cells are both processes that are found altered in Duchenne's muscular dystrophy, thus representing one of the most severe causes underlying an inefficient muscle tissue regeneration, thus contributing to disease etiology and progression [36–38].

2.4 The role of the endocannabinoid system in Duchenne's muscular dystrophy

Among the hereditary myopathies, Duchenne's muscle dystrophy (DMD) represents the most frequent one, affecting predominantly young boys with a frequency of approximately 1:3500. Mutations in the X-linked gene encoding for the structural protein dystrophin, which plays a key structural role by physically linking the cytoskeleton to the surrounding extracellular matrix through the cell membrane, are the cause of the disease. The most frequent mutations are large intragenic deletions (65% of the cases), intragenic duplications (6–10% of the cases), or point mutations associated to other sequence variations (30–35% of the cases). Dysfunctional dystrophin leads to progressive and irreversible loss of muscle function [39–42].

As briefly mentioned earlier, it has been recently demonstrated that the lack of functional dystrophin is also the primary cause of an asymmetric cell division, altered morphogenesis, and inefficient differentiation of satellite cells, the muscle stem cells normally deputed to regenerate injured muscle fibers [36, 38]. Surprisingly, the number of satellite cells was found significantly increased in both human and murine skeletal muscles affected by DMD. Thus, while at the early stage of DMD, satellite cell-mediated muscle regeneration is able to attenuate degeneration; at later stages of disease progression, this process is inefficient [37, 43].

Therefore, in the light of these findings as well as the prominent role played by ECS during skeletal muscle cell proliferation earlier described, my research group has explored whether the cannabinoids might represent a promising alternative approach to treat skeletal muscle disorders including DMD.

Toward this goal, we have recently characterized the expression profile of the ECS in whole muscles and isolated myoblasts of both mice and patients affected by DMD. No statistically significant differences were found in 2-AG levels between healthy and DMD donors, while higher levels of 2-AG were found in the muscles of 3-weeks-old mdx mice. However, 2-AG levels in both mdx mouse quadriceps and gastrocnemius, and in control gastrocnemius, first decreased (from 3 to 5 weeks of age) and then increased (from 5 to 8 weeks of age). Interestingly, 2-AG levels in the gastrocnemius were higher in mdx mice at 8 weeks and then 3 and 5 weeks.

Furthermore, in agreement with previous studies [36, 37, 43], we found that the total number of satellite cells isolated from skeletal muscles of mdx mice was significantly higher than in control mice. Intriguingly, by means of quantitative qPCR (qPCR) and RNA-Seq analysis, we demonstrated that the lack of dystrophin was accompanied by a significant increase in the transcript levels of CB1, occurring exclusively in satellite cells but not in other muscle resident cells including fibro-adipogenic progenitor (FAP) or macrophage cells. Thus, we provided evidence that in both human and murine skeletal muscles affected by DMD occur significant changes in 2-AG levels. These changes are associated with an increased expression of CB1 gene and concomitant increase in the number of satellite cells [44].

In addition to these findings, we have also demonstrated that PAX7, the most known master gene regulating satellite cell activation and self-renewal [45, 46], underwent changes very similar to CB1 showing a bell-shaped profile with the highest degree of expression at DMD onset and declining then over time. Intriguingly, by the use of bioinformatics and biochemical analyses, it was demonstrated that PAX7 directly binds and upregulates the CB1 gene in dystrophic more than in healthy muscles. In summary, the incorrect PAX7-CB1 cross talk, causing an excessive satellite cell proliferation and reduction of differentiation, was uncovered as a new target mechanism to treat DMD. Antagonism of CB1 receptors by rimonabant, opposite to their activation by ACEA, reduces human satellite cell proliferation and enhances the formation of myotubes from either satellite cells from healthy tissue or human myoblasts from DMD patients. Furthermore, in dystrophic mdx mice, the acute (2 weeks) or prolonged (12 weeks) treatment with rimonabant significantly prevented the loss of muscle coordination and strength compared to control (not treated) mice. Biochemical and histological analyses of mdx mice at the end of treatment revealed that the effect of rimonabant was associated with an increased number of healthy/regenerating fibers and decreased tissue levels of inflammatory markers including interleukin-6 receptor (IL-6R), tumor necrosis factor- α (TNF α), transforming growth factor β (TGF- β), and inducible nitric oxide synthase (iNOS) (Figure 2).

2.5 The use of plant-derived cannabinoids in Duchenne's muscular dystrophy

In addition to endocannabinoids, our research group has also explored the use of plant-derived cannabinoids (or phytocannabinoids) to treat DMD. Phytocannabinoids encompass a group of numerous compounds present in *Cannabis sativa*. Due to its euphoric properties, Δ^9 -tetrahydrocannabinol (THC) is the best-known constituent of *Cannabis* [47]. However, many other phytocannabinoids (more than 100) have been purified and chemically characterized [48]. Among them, cannabidiol (CBD) and its analog cannabivarin (CBDV), contrary to Δ^9 -THC, do not induce euphoric effects and showed their efficacy in a considerable number of preclinical as well as clinical studies [49–52]. Besides CBD and CBDV, *Cannabis sativa* may also contain up to 50% of Δ^9 -tetrahydrocannabivarin (THCV), the propyl side chain analog of THC ([53, 54]. THCV is also undergoing clinical evaluation for the treatment of metabolic disorders [55]. In differentiating

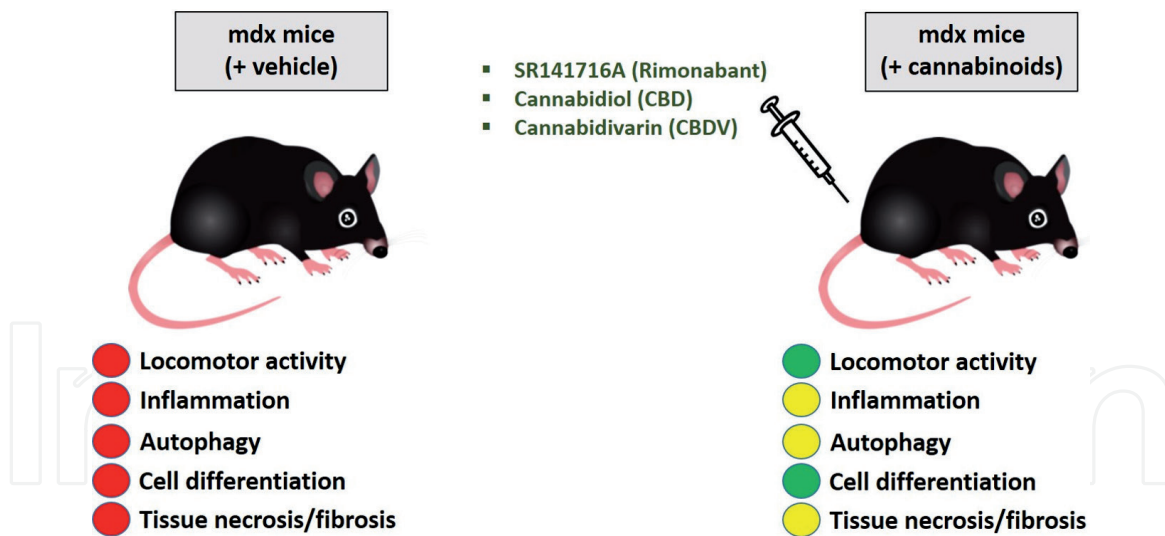


Figure 2.
Graphical representation showing the effects of cannabinoids in mdx mice.

C2C12 myoblasts, we found that the CBD (1 μ M) and CBDV (1 and 3 μ M), but not THCv, significantly promoted the myotube formation. Intriguingly, this latter effect was more pronounced following acute (5, 15 min and 3 hours) rather than the prolonged (72 hours) exposure and associated with a transient elevation of $[Ca^{2+}]_i$ in a manner dependent on TRPV1 channel activation. The efficacy of CBD and CBDV has been then demonstrated in vivo. In mdx mice of 5 weeks, the most used animal model of DMD [56, 57], we found that the locomotor activity measured by means of three different tests (rotarod, weight, and forelimb grip strength) was rescued completely when compared to control vehicle-treated mice. Of note, the effect of CBD and CBDV in mdx mice was not only found at 7 weeks (a time near the disease onset) but also at 34 weeks, when the muscle damage had further progressed. Interestingly, the beneficial effects of the phytocannabinoids were associated with a local or systemic anti-inflammatory effect and restoration of autophagy, two pathophysiological features of DMD (**Figure 2**) [36, 58]. However, in our recent published study, we did not perform a detailed time-course experiments; therefore, we cannot draw any conclusion as to whether the anti-inflammatory effects of the phytocannabinoids are the cause or the consequence of their pro-autophagic actions nor on whether at different stages of the disease; these compounds may affect myoblast differentiation. On the other hand, the finding of their anti-inflammatory and pro-autophagic actions opens the possibility of testing CBD and CBDV as add-on therapeutics to other agents that are currently undergoing clinical trials in DMD, such as exon-skipping agents [59]. However, it is worth mentioning that mdx mice do not model appropriately all aspects of the human DMD disease. Therefore, while beyond the scope of the present investigation, future studies in more suitable models, such as the golden retriever dog model [56, 57], are needed in order to suggest the therapeutic use of CBD and CBDV in DMD. In primary human satellite cells, CBD and CBDV and also THCv were capable of enhancing differentiation. Most importantly similar were also obtained in differentiating myoblasts isolated from seven different donors (ranging from 1 to 7 years old) diagnosed with DMD. Thus, these results potentially extended the therapeutic usefulness of these compounds at counteracting dystrophies. Interestingly, in satellite cells, the target through which these compounds produce their differentiating effect appears to be different from that mediating this effect in murine C2C12 myoblasts. In fact, TRPA1, rather than TRPV1, channels mediated an elevation of $[Ca^{2+}]_i$.

3. Conclusions

Inherited muscular dystrophies originating from mutations in one of the components forming the dystrophin glycoprotein complex show specific features but similar clinical characteristics. Albeit controversial, no overt clinical improvement was observed in patients with DMD under current therapies. For this reason, the identification of therapies capable of alleviating the progression of the disease is imperative. There are emerging evidences that the pharmacological regulation of ECS activity as well as the use of certain phytocannabinoids may attenuate the progressive and irreversible loss of muscle function. In conclusion, there is hope that the use of cannabinoids in DMD could represent a keystone to open new fields of research in discovering novel mechanisms able to preserve muscle tissue activity by preserving its integrity or promoting its regeneration.

Conflict of interest

I confirm there are no conflicts of interest.

IntechOpen

Author details

Fabio Arturo Iannotti
Endocannabinoid Research Group, Institute of Biomolecular Chemistry (ICB),
National Research Council (CNR), Pozzuoli, NA, Italy

*Address all correspondence to: fabio.iannotti@icb.cnr.it

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nature Reviews. Drug Discovery*. 2004;**3**:771-784
- [2] Di Marzo V, De Petrocellis L. Why do cannabinoid receptors have more than one endogenous ligand? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2012;**367**:3216-3228
- [3] Iannotti FA, Piscitelli F, Martella A, Mazzarella E, Allarà M, Palmieri V, et al. Analysis of the "endocannabinoidome" in peripheral tissues of obese Zucker rats. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*. 2013;**89**:127-135
- [4] Iannotti FA, Di Marzo V, Petrosino S. Endocannabinoids and endocannabinoid-related mediators: Targets, metabolism and role in neurological disorders. *Progress in Lipid Research*. 2016;**62**:107-128
- [5] Baggelaar MP, Maccarrone M, van der Stelt M. 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Progress in Lipid Research*. 2018;**71**:1-17
- [6] Di Marzo V, Wang J. *Endocannabinoidome: The World of Endocannabinoids and Related Mediators*. Academic Press; 19 September 2014. ISBN: 9780128004296, ISBN: 9780124201262
- [7] Gatta-Cherifi B, Cota D. New insights on the role of the endocannabinoid system in the regulation of energy balance. *International Journal of Obesity*. 2016;**40**:210-219
- [8] Perkins JM, Davis SN. Endocannabinoid system overactivity and the metabolic syndrome: Prospects for treatment. *Current Diabetes Reports*. 2008;**8**:12-19
- [9] Di Marzo V. CB1 receptor antagonism: Biological basis for metabolic effects. *Drug Discovery Today*. 2008;**13**:1026-1041
- [10] Di Marzo V. The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia*. 2008;**51**:1356-1367
- [11] Bisogno T, Di Marzo V. Cannabinoid receptors and endocannabinoids: Role in neuroinflammatory and neurodegenerative disorders. *CNS & Neurological Disorders Drug Targets*. 2010;**9**:564-573
- [12] Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters*. 1994;**350**:240-244
- [13] Liu YL, Connoley IP, Wilson CA, Stock MJ. Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep ob/Lep ob mice. *International Journal of Obesity*. 2005;**29**:183-187
- [14] Cavuoto P, McAinch AJ, Hatzinikolas G, Janovská A, Game P, Wittert GA. The expression of receptors for endocannabinoids in human and rodent skeletal muscle. *Biochemical and Biophysical Research Communications*. 2007;**364**:105-110
- [15] Cavuoto P, McAinch AJ, Hatzinikolas G, Cameron-Smith D, Wittert GA. Effects of cannabinoid receptors on skeletal muscle oxidative pathways. *Molecular and Cellular Endocrinology*. 2007;**267**:63-69
- [16] Esposito I, Proto MC, Gaggero P, Laezza C, Miele C, Alberobello AT,

- et al. The cannabinoid CB1 receptor antagonist rimonabant stimulates 2-deoxyglucose uptake in skeletal muscle cells by regulating the expression of phosphatidylinositol-3-kinase. *Molecular Pharmacology*. 2008;**74**:1678-1686
- [17] Eckardt K, Sell H, Taube A, Koenen M, Platzbecker B, Cramer A, et al. Cannabinoid type 1 receptors in human skeletal muscle cells participate in the negative crosstalk between fat and muscle. *Diabetologia*. 2009;**52**:664-674
- [18] Lipina C, Stretton C, Hastings S, Hundal JS, Mackie K, Irving AJ, et al. Regulation of MAP kinase-directed mitogenic and protein kinase B-mediated signaling by cannabinoid receptor type 1 in skeletal muscle cells. *Diabetes*. 2010;**59**:375-385
- [19] Heyman E, Gamelin F-X, Aucouturier J, Di Marzo V. The role of the endocannabinoid system in skeletal muscle and metabolic adaptations to exercise: Potential implications for the treatment of obesity. *Obesity Reviews*. 2012;**13**:1110-1124
- [20] Sparling PB, Giuffrida A, Piomelli D, Roskopf L, Dietrich A. Exercise activates the endocannabinoid system. *NeuroReport*. 2003;**14**:2209-2211
- [21] Feuerecker M, Hauer D, Toth R, Demetz F, Hölzl J, Thiel M, et al. Effects of exercise stress on the endocannabinoid system in humans under field conditions. *European Journal of Applied Physiology*. 2012;**112**:2777-2781
- [22] Heyman E, Gamelin F-X, Goekint M, Piscitelli F, Roelands B, Leclair E, et al. Intense exercise increases circulating endocannabinoid and BDNF levels in humans—Possible implications for reward and depression. *Psychoneuroendocrinology*. 2012;**37**:844-851
- [23] Raichlen DA, Foster AD, Seillier A, Giuffrida A, Gerdeman GL. Exercise-induced endocannabinoid signaling is modulated by intensity. *European Journal of Applied Physiology*. 2013;**113**:869-875
- [24] Keeney BK, Meek TH, Middleton KM, Holness LF, Garland T. Sex differences in cannabinoid receptor-1 (CB1) pharmacology in mice selectively bred for high voluntary wheel-running behavior. *Pharmacology, Biochemistry, and Behavior*. 2012;**101**:528-537
- [25] Dietrich A, McDaniel WF. Endocannabinoids and exercise. *British Journal of Sports Medicine*. 2004;**38**:536-541
- [26] Keeney BK, Raichlen DA, Meek TH, Wijeratne RS, Middleton KM, Gerdeman GL, et al. Differential response to a selective cannabinoid receptor antagonist (SR141716: Rimonabant) in female mice from lines selectively bred for high voluntary wheel-running behaviour. *Behavioural Pharmacology*. 2008;**19**:812-820
- [27] Hill MN, Titterness AK, Morrish AC, Carrier EJ, Lee TT-Y, Gil-Mohapel J, et al. Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. *Hippocampus*. 2009;**20**:NA-NA
- [28] Hill MN, Kambo JS, Sun JC, Gorzalka BB, Galea LAM. Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours. *The European Journal of Neuroscience*. 2006;**24**:1845-1849
- [29] Dubreucq S, Durand A, Matias I, Bénard G, Richard E, Soria-Gomez E, et al. Ventral tegmental area cannabinoid type-1 receptors control voluntary exercise performance. *Biological Psychiatry*. 2013;**73**:895-903
- [30] Gamelin F-X, Aucouturier J, Iannotti FA, Piscitelli F, Mazzarella E,

- Aveta T, et al. Exercise training and high-fat diet elicit endocannabinoid system modifications in the rat hypothalamus and hippocampus. *Journal of Physiology and Biochemistry*. 2016;**73**:335-347
- [31] Thompson Z, Argueta D, Garland T, DiPatrizio N. Circulating levels of endocannabinoids respond acutely to voluntary exercise, are altered in mice selectively bred for high voluntary wheel running, and differ between the sexes. *Physiology & Behavior*. 2017;**170**:141-150
- [32] Hutchins-Wiese HL, Li Y, Hannon K, Watkins BA. Hind limb suspension and long-chain omega-3 PUFA increase mRNA endocannabinoid system levels in skeletal muscle. *The Journal of Nutritional Biochemistry*. 2012;**23**:986-993
- [33] Iannotti FA, Silvestri C, Mazzarella E, Martella A, Calvigioni D, Piscitelli F, et al. The endocannabinoid 2-AG controls skeletal muscle cell differentiation via CB1 receptor-dependent inhibition of Kv7 channels. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**:E2472-E2481
- [34] Iannotti FA, Barrese V, Formisano L, Miceli F, Tagliatela M. Specification of skeletal muscle differentiation by repressor element-1 silencing transcription factor (REST)-regulated Kv7.4 potassium channels. *Molecular Biology of the Cell*. 2013;**24**:274-284
- [35] Iannotti FA, Panza E, Barrese V, Viggiano D, Soldovieri MV, Tagliatela M. Expression, localization, and pharmacological role of Kv7 potassium channels in skeletal muscle proliferation, differentiation, and survival after myotoxic insults. *The Journal of Pharmacology and Experimental Therapeutics*. 2010;**332**:811-820
- [36] Dumont NA, Wang YX, Von Maltzahn J, Pasut A, Bentzinger CF, Brun CE, et al. Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nature Medicine*. 2015;**21**:1455-1463
- [37] Kottlors M, Kirschner J. Elevated satellite cell number in Duchenne muscular dystrophy. *Cell and Tissue Research*. 2010;**340**:541-548
- [38] Chang NC, Chevalier FP, Rudnicki MA. Satellite cells in muscular dystrophy-lost in polarity. *Trends in Molecular Medicine*. 2016;**22**:479-496
- [39] Falzarano M, Scotton C, Passarelli C, Ferlini A. Duchenne muscular dystrophy: From diagnosis to therapy. *Molecules*. 2015;**20**:18168-18184
- [40] Nallamilli BRR, Ankala A, Hegde M. Molecular diagnosis of duchenne muscular dystrophy. *Current Protocols in Human Genetics*. 2014;**83**:9.25.1-9.25.29
- [41] Govoni A, Magri F, Brajkovic S, Zanetta C, Faravelli I, Corti S, et al. Ongoing therapeutic trials and outcome measures for Duchenne muscular dystrophy. *Cellular and Molecular Life Sciences*. 2013;**70**:4585-4602
- [42] Magri F, Govoni A, D'Angelo MG, Del Bo R, Ghezzi S, Sandra G, et al. et al., Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. *Journal of Neurology*. 2011;**258**:1610-1623
- [43] Jiang C, Wen Y, Kuroda K, Hannon K, Rudnicki MA, Kuang S. Notch signaling deficiency underlies age-dependent depletion of satellite cells in muscular dystrophy. *Disease Models & Mechanisms*. 2014;**7**:997-1004
- [44] Iannotti FA, Pagano E, Guardiola O, Adinolfi S, Saccone V, Consalvi S, et al. Genetic and pharmacological regulation

- of the endocannabinoid CB1 receptor in Duchenne muscular dystrophy. *Nature Communications*. 2018;**9**:3950
- [45] Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. Pax7 is required for the specification of myogenic satellite cells. *Cell*. 2000;**102**:777-786
- [46] Reimann J, Brimah K, Schröder R, Wernig A, Beauchamp JR, Partridge TA. Pax7 distribution in human skeletal muscle biopsies and myogenic tissue cultures. *Cell and Tissue Research*. 2004;**315**:233-242
- [47] Di Marzo V, Piscitelli F. The endocannabinoid system and its modulation by phytocannabinoids. *Neurotherapeutics*. 2015;**12**:692-698
- [48] Morales P, Hurst DP, Reggio PH. Molecular targets of the phytocannabinoids: A complex picture. *Progress in the Chemistry of Organic Natural Products*. 2017;**103**:103-131
- [49] Burstein SH, Zurier RB. Cannabinoids, endocannabinoids, and related analogs in inflammation. *The AAPS Journal*. 2009;**11**:109-119
- [50] Hill AJ, Williams CM, Whalley BJ, Stephens GJ. Phytocannabinoids as novel therapeutic agents in CNS disorders. *Pharmacology & Therapeutics*. 2012;**133**:79-97
- [51] Fernández-Ruiz J, Moro MA, Martínez-Orgado J. Cannabinoids in neurodegenerative disorders and stroke/brain trauma: From preclinical models to clinical applications. *Neurotherapeutics*. 2015;**12**:793-806
- [52] Feliú A, Moreno-Martet M, Mecha M, Carrillo-Salinas FJ, de Lago E, Fernández-Ruiz J, et al. A Sativex®-like combination of phytocannabinoids as a disease-modifying therapy in a viral model of multiple sclerosis. *British Journal of Pharmacology*. 2015;**172**:3579-3595
- [53] Fishedick JT. Identification of terpenoid chemotypes among high (-)-trans- Δ^9 -tetrahydrocannabinol-producing *Cannabis sativa L. Cultivars*. *Cannabis and Cannabinoid Research*. 2017;**2**:34-47
- [54] McPartland JM, Duncan M, Di Marzo V, Pertwee RG. Are cannabidiol and Δ^9 -tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *British Journal of Pharmacology*. 2015;**172**:737-753
- [55] Jadoon KA, Ratcliffe SH, Barrett DA, Thomas EL, Stott C, Bell JD, et al. Efficacy and safety of cannabidiol and tetrahydrocannabivarin on glycemic and lipid parameters in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled, parallel group pilot study. *Diabetes Care*. 2016;**39**:1777-1786
- [56] Collins CA, Morgan JE. Duchenne's muscular dystrophy: Animal models used to investigate pathogenesis and develop therapeutic strategies. *International Journal of Experimental Pathology*. 2003;**84**:165-172
- [57] McGreevy JW, Hakim CH, McIntosh MA, Duan D. Animal models of Duchenne muscular dystrophy: From basic mechanisms to gene therapy. *Disease Models & Mechanisms*. 2015;**8**:195-213
- [58] Fiacco E, Castagnetti F, Bianconi V, Madaro L, De Bardi M, Nazio F, et al. Autophagy regulates satellite cell ability to regenerate normal and dystrophic muscles. *Cell Death and Differentiation*. 2016;**23**:1839-1849
- [59] Nakamura A. Moving towards successful exon-skipping therapy for Duchenne muscular dystrophy. *Journal of Human Genetics*. 2017;**62**:871-876