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

6,300

Open access books available

172,000

International authors and editors

190M

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

Neuroprotective Properties of Cannabinoids in Cellular and Animal Models: Hypotheses and Facts

Lucas D. Udovin, Andrea Aguilar, Tamara Kobiec, María I. Herrera, Santiago Perez Lloret, Nicolás Toro Urrego and Rodolfo A. Kölliker Frers

Abstract

Progressive neuronal loss is a typical characteristic of neurodegenerative diseases. In Parkinson's disease, the loss of dopaminergic neurons in the basal ganglia results in impaired mobility and flawed muscle control. The loss of cholinergic neurons largely in the basal forebrain contributes to memory and attention deficits and the overall cognitive impairment in Alzheimer's disease. This being said, neuroprotective drugs should be expected to preserve and/or restore the functions affected by neuronal loss, and substantially prevent cell death. The endocannabinoid system, comprising lipid mediators able to bind to and activate cannabinoid receptors, has emerged as a therapeutic target of potential interest in a variety of central nervous system diseases. Palmitoylethanolamide (PEA) is one of the most important endocannabinoids, which has a key role in modulating oxidative stress and inflammatory response with neuroprotective potential in neurological disorders. Neurodegenerative diseases undergo varied, progressive stages. The current therapeutical approaches are beginning to fall short when it comes to meet the expected results, urging to either develop or identify or develop new effective treatments. This chapter discusses the neuroprotective potential of new drugs, aiming to shed some light on their proposed mechanism of action and their effect in cellular and animal models of neurodegeneration.

Keywords: Parkinson's disease, Alzheimer's disease, endocannabinoids, palmitoylethanolamide (PEA), dopaminergic neurons

1. Introduction

The palmitoylethanolamide (PEA) is an endogenous biologically active lipids belonging to the family of the endogenous cannabinoid. PEA has many uses in a range of therapeutics areas, such as: neurological diseases, neurodegeneration, and pain.

Several studies have been carried out to define the molecular mechanism of PEA. However, at time, it was proposed that the existence of a mechanism

Figure 1.

Scheme of the performance of the PEA through the union to its different receptors. The PEA, through PPAR α , mainly performs metabolic functions such as the regulation of lipid metabolism and exerts neuroprotective functions due to the reduction of transcription of various proinflammatory cytokines. After the binding of PEA to PPAR α in the nucleus, it heterodimerizes with the RXR receptor, to then join specific DNA sequences called PPRE. These PPRE modulate transcription of target genes that control a wide variety of activities metabolic and physiological. On the other hand, it has been hypothesized that the PEA can also exercise its biological functions through different transmembrane receptors like GPR55, GPR119, and TRPV1, although this issue is still controversial.

receptor-dependent, and several studies demonstrated that PEA can act via direct activation of two different receptors: the orphan GPCR 55 (GPR55) [1] and the PPAR- α [2]. It was discovered that there is a wide variety receptors capable of interacting with PEA. All of them are belonged to these two receptors families, **Figure 1**.

Other supposition postulated that PEA could also be a cannabinoid receptor type 2 (CB2) receptor agonist; however, studies suggested that it has very weak affinity for this receptor [3]. In other hand, the transient receptor potential vanilloid receptor type 1 (TRPV1) channels can be activated for PEA in an indirectly way, **Figure 1**. These receptors are important targets of many endocannabinoids.

All data, which will be shown in this chapter, suggest that the action mechanism of PEA operates for several different ways. In the central and the peripheral nervous system, these mechanisms have collaborative interactions essential for the most important therapeutic effects of PEA.

2. Receptors of PEA

GPR55 and PPAR- α are the two most important receptors for PEA. GPR55 is a receptor belongs to the large family of GPCRs. It is expressed in brain areas, including the hippocampus, striatum, cortex, forebrain, and cerebellum. It has been reported that GPR55 utilizes the high concentration of intracellular to trigger a cascade of signalling events [4]. NF- κ B, cAMP, MAPK, ERK1/2, and transcriptional regulators such as nuclear factor of activated T-cells (NFAT) are involved to GPR55 activation [5]. Other receptor belongs to the family of GPCR is GPR119, which can recognize oleoylethanolamide (OEA) and PEA. However, these two acylethanolamides do not interact with classical cannabinoid receptors such as CB1 and CB2 [6].

PPAR- α belongs to the family of PPARs and acts as a nuclear receptor protein. PPAR- α is present in many tissues and organs; liver, intestine, heart, muscle,

brain, kidney, and adipose tissue. Also, this receptor is present in cells of the immune system PPAR- α . Their main functions are involved in the control of inflammatory processes and in the transcription factor regulating gene expression. In the same way, it is accepted that the binding of PEA to PPAR- α induces a heterodimerization event with the retinoic acid receptor (RXR), forming the activated receptor complex, which decrease the transcription of pro-inflammatory genes [7].

Both receptors have recently emerged as a putative target for the treatment of pain, inflammation, and neurodegenerative diseases [8].

3. Other components involved indirectly in the mechanism of PEA

TRPV1 channel belongs to a subfamily of transient receptor potential channels (TRP channels). It is called 'the capsaicin receptor.' This is conformed by intramembrane loop linking the transmembrane domains, forming the pore channel region [9]. TRPV1 is present in sensory nerve fibres and dorsal root ganglia, keratinocytes, in brain neurons, and other cell types [10–13]. TRPV1 is activated by stimulation of the non-selective ion channel, permeable to cations. Also, it is activated by exogenous or endogenous chemical compounds [9, 14].

The changes in the phosphorylation state of TRPV1 induced by regulatory proteins (including PKA, PKC, ATP, phosphoinositide binding protein (PIRT) and phosphatidylinositol 4,5-bisphosphate (PIP2)) influent in the function of the receptor [15, 16]. The changes in the phosphorylation state produce an activation of TRPV1, then this trigger the signaling cascade to pain transmission, neurotoxicity, and inflammation [13, 17]. The high concentration of intracellular Ca^{2+} produces the stimulation of two processes very important. On the one hand, the stabilization of the channel by locked conformational. On the other hand, the inactivation of TRPV1 channel by Ca^{2+} -dependent phosphatases, such as calcineurin, which dephosphorylate it [15, 16]. This process contributes to the anti-inflammatory and analgesic actions of TRPV1 [16, 17].

There are many hypotheses about the mechanisms for the action of PEA with TRPV1 channels. One of them proposes that TRPV1 channels can be indirectly activate via PPAR- α due the action of PEA. Other mechanism proposes an indirectly activation of TRPV1 through the allosteric effects produce for PEA. It could increase AEA - or 2-AG induced activation and desensitization at TRPV1 channels [10, 18, 19].

The cannabinoid receptors types 1 and 2 (CB1 and CB2) are members of the G protein coupled receptor (GPCR) family that were identified over 20 years ago. [20, 21]. The CB1 receptor is often expressed in the brain, in the peripheral nervous system and presynaptic terminals. Also, it is expressed in almost all mammalian tissue and organs [22]. Its activation usually inhibits neurotransmitter release; the CB1 activation inhibits adenylate cyclase activity with the subsequent stimulates MAPK activity or reduction of intracellular levels of cAMP [23]. Consequently to the coupling of CB1 to PKB (Akt), phosphoinositide 3-kinase and PLC/inositol1, 4,5-trisphosphate/PKC (PLC β /IP3/PKC) pathways [24, 25].

The CB2 receptors are involved in the activated astrocytes and microglia in the brain, where are expressed in low concentration [26]. However, this receptor is expressed in peripheral organs and cells of the immune system [16, 27–29]. One of the most important functions of the CB2 receptor is controlling the inflammatory responses [16, 30]. The CB2 receptor activation promotes MAPK activity and inhibits adenylate cyclase activity [31].

CB1 and CB2 can be indirectly activated by PEA through several of the mechanisms, although they are not direct targets [3, 12, 19, 32].

4. Brain injury and hypoxia ischemia

There are different causes of brain injury. However, none of them have a specific and efficient treatment to reverse its effects. The hypoxia-ischemia (HI) is one of the most important causes of neuronal damage and this will be the focus of this section.

HI impairs normal blood flow and reduces the oxygen and glucose supply to cells. This affects the cellular energy demand through alterations of aerobic metabolism, mitochondrial oxidative phosphorylation, and the anaerobic glycolysis [33]. Even though a HI event affects the heart, liver, kidneys, gut, and every other organ, it poses the central nervous system (CNS) to severe danger, as shown in human and animal models [33, 34]. The mechanism of brain injury involves a series of events referred to as the “excito-oxidative cascade”; it triggers the activation of excitatory glutamate receptors leading to excessive neuronal calcium influx. Calcium flooding through NMDA channels activates nitric oxide synthase raising the concentration of the free radical nitric oxide to toxic levels. The increase of both nitrogen- and oxygen-free radical species generated during the post-hypoxic re-oxygenation phase impairs the function of the enzymes associated with oxidative phosphorylation

Hypoxia-ischemia		
Model	Global	Focal
Animal (mouse/rat)	<ul style="list-style-type: none">• Bilateral common carotid artery (CCA) occlusion combined with systemic hypotension, hypoxia or anoxia: this model results in variable ischemia depending on how long hypotension, hypoxia or anoxia last in neonatal rats	<ul style="list-style-type: none">• Permanent middle cerebral artery (MCA) occlusion: electrocauterization of the MCA proximal to the origin of the lateral lenticulostriate arteries
	<ul style="list-style-type: none">• Four vessel occlusions: A cauterizing needle causes blood electrocoagulation in the vertebral arteries, with clamps around the CCAs. The experimenter manipulates the clamps through an incision in the neck of adult rats, tightening the clamps 24 h later	<ul style="list-style-type: none">• Transient middle cerebral artery (MCA) occlusion: Ligation with surgical clips or sutures
	<ul style="list-style-type: none">• Uterus horns immersion: It consists of removing uterus horns containing fetuses from ready-to-deliver rats and immersing them in a water bath at 37°C for 5–20 min. The degree of HI is proportional to the immersion span	<ul style="list-style-type: none">• Non-invasive model using intraluminal sutures: It consists of inserting a nylon filament through the proximal external carotid artery to occlude the MCA. Reperfusion follows later suture removal
Cell culture	<ul style="list-style-type: none">• Cell culture models. There are a few cell cultures models that mimic the hypoxic–ischemic injury. Among them, oxygen and glucose deprivation (OGD) is a widely used in vitro model of ischemia that results in apoptotic and necrotic cell death	

Table 1.
Animals and cellular models to hypoxia-ischemia: in the table are showed the most used models for global and focal hypoxia ischemia.

and electron transport. Calcium toxicity is also mediated by activation of other enzymes including caspases, calpains and other proteases, and lipases, which attack mitochondria and other cellular machinery. As a result, the damaged mitochondria release signals leading to apoptosis or programmed cell death as long as the energy supplies persist, and otherwise to necrosis and destruction of cellular membranes when energy becomes exhausted. Impairment of mitochondrial oxidative phosphorylation results in lactic acid accumulation, which may be less toxic in the neonatal brain compared with the adult brain.

The extent of damage to the central nervous system determines whether global or focal HI takes place. The focal hypoxia-ischemia occurs when an end artery is exclusively occluded. However, the spread of damage depends on the duration and degree of the occlusion and on how well the collateral irrigation copes with metabolic demands. In two brain areas, the core and the penumbra are found the most evident effect of damage. In the core area, the collateral blood supply is reduced and shows the most severe and irreversible lesions. The penumbra surrounds the core area and receives collateral irrigation, reducing its vulnerability to the occlusion. Consequently, penumbra cells either recover from the transient damage or start a series of events that ultimately lead to cell death [35].

Global cerebral HI remains a superlative cause of perinatal brain injury, ultimately leading to neurologic dysfunction, which becomes manifest as cerebral palsy, mental retardation, and epilepsy [36, 37]. Encephalopathy following perinatal HI occurs in 1–3 per 1000 term births in the United Kingdom [38]. Similarly, stroke affects 15 million people worldwide each year and is the leading cause of disability in the United States of America [39].

Different models are used to investigate the pathophysiology of HI. Even though none of them strictly reproduce the clinical conditions, however, they are used to study cellular and molecular mechanisms underlying HI and test the potential therapeutic agents, **Table 1**.

5. PEA and Hypoxia—Ischemia

From the 1970s, our understanding of the pathogenesis of the hypoxia-ischemia (HI) brain injury has grown considerably [40], both in the physiological and molecular aspects. This progress has taken us closer to develop different therapies to prevent or minimize neuronal HI damage. In this regard, the hypothermia, nitric oxide synthase inhibitors, and neuronal growth factors that help neuronal survival and may play a role in rescuing brain tissue and promoting brain growth following injury and blocking HI apoptosis by caspase inhibition [36]. Hypothermia is one of the few strategies applied in the clinical treatment of perinatal brain injury due to both HI and stroke [41]. With the advance in molecular science, pharmacology, and genomics, new therapeutic approaches are arising. Accordingly, pharmacogenomics has gathered the interest of many groups allowing individualized treatment and maximizing therapeutic effects while minimizing side effects [42].

The treatment with PEA has been used in cellular and animal models. Rats were exposed to neonatal anoxia-ischemia (AI) and then treated with vehicle or PEA showed that neonatal AI was associated with decreased locomotion, as well as recognition and spatial memory impairments. Furthermore, these deficits were accompanied with enhanced neuroinflammation and astrogliosis, as well as a decreased PPAR α expression. PEA treatment was able to prevent neuroinflammation, reduce astrogliosis, and preserve cognitive functions [43]. In addition, it has also been shown that in a mice model of perinatal hypoxic-ischemic (HI) encephalopathy, PEA exert neuroprotective effects on cultured cortical neurons

being mediated by TRPV4 receptor, and cotreatment with OEA and PEA is able to enhance neuroprotective effects of the acylethanolamides [18]. A neuroprotective function of injected PEA and OEA has been substantiated in mice with transient middle cerebral artery occlusion [44, 45], but both in the OEA and in the PEA, the mechanism by which they would be exercising their neuroprotective actions would be through PPAR α activation [44]. Besides, PEA may potentiate microglial cell motility after focal cerebral ischemia by an apparent non-cannabinoid receptor-mediated mechanism [46]. PEA exerts neuroprotection and reduces inflammatory secondary events associated with brain ischemia reperfusion injury (middle cerebral artery occlusion (MCAo)), in rats subjected to MCAo and treated with PEA in conjunction with Luteolin post-ischemia showed reduced edema and brain infarct volume, improved neurobehavioral functions, and reduced expression of pro-inflammatory markers and astrocyte markers. In the same sense, a cohort of 250 stroke patients undergoing neurorehabilitation on either an inpatient or an outpatient basis that were treated for 60 days with a pharmaceutical preparation of PEA and luteolin improved the neurological status, impairment of cognitive abilities, the degree of spasticity, pain, and independence in daily living activities [47]. PEA-mediated improvements in tissues histology shown by reduction of lesion size and improvement in apoptosis level (assayed by Bax and Bcl-2) further support the efficacy of PEA therapy in MCAo mice. PEA treatment blocked infiltration of astrocytes and restored MCAo-mediated reduced expression of PAR, nitrotyrosine, iNOS, chymase, tryptase, growth factors (BDNF and GDNF), and GFAP and also inhibited the MCAo-mediated increased expression of pJNK, NF-kB, and degradation of I κ B- α , as well as improved neurobehavioral functions as evaluated by motor deficits [48]. In perinatal asphyxia (PA) murine model, PEA attenuated PA-induced cellular and molecular hippocampal damage and its corresponding behavioral alterations. PEA treatment improved dendritic cytoskeleton alteration in CA1 hippocampal area evidenced by the increase of MAP-2 and the reduction of pNF-H/M immunostaining and protein expression levels, as well as, vertical exploration impairments and anxiety-related behaviors [49]. On the other hand, in a cellular oxygen and glucose deprivation (OGD) model that mimics brain-ischemia, PEA and luteolin pre-treatment synergistically prevented the OGD-induced degranulation of mast cells and reduced the neurotoxic potential of MC/9 cells conditioned medium as well as pure neurons susceptibility to OGD [50].

6. PEA and neurodegenerative diseases

Many neurodegenerative diseases are characterized by loss of the functions of the nervous system causes for neuronal disorders or damage. The most frequently diseases are Alzheimer's disease (AD) and Parkinson's disease (PD). The effect of the damage can lead to loss of the mobility, behavioral disorders, and dementia.

The effects of PEA were demonstrated in several assays. To AD, for example, it was registered in a mouse model, that administration of the PEA produces a reduction in the behavioral impairments [51].

In assays *in vivo* using adults' male rats, which consisted in the intrahippocampal injection of amyloid- β 1–42 (A β 1–42) peptide, the administration of PEA affected in: the high transcription and expression of GFAP and S100 β (activators of astrocytes), the increased expression of BACE1 and APP (amyloidogenic), and phosphorylated τ proteins. Also, PEA changed the altered expression of microtubule-associated protein (MAP-2) and cognitive functions induced [52].

An animal PD model that use the injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been showed the neuroprotective

actions of PEA [53]. A treatment with PEA neutralizes the activation of astrocytes and expression of iNOS protein, the loss of nigrostriatal neurons, and the expression of microtubule-associated, produced by MPTP in the model. In other hand, PEA decreased the motor dysfunctions associated to the MPTP effects. The activation of PPAR- α was a key piece in the PEA effects [53]. On the other hand, in cellular models, PEA has been shown to have protective effect on SH-SY5Y neuroblastoma against 6-OHDA damage, partly by inhibiting endoplasmic reticulum stress detrimental response [54].

Therapeutic effects of PEA have also been reported in several chronic models of MS, such as chronic relapsing experimental autoimmune encephalomyelitis (CREAE), Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), and myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE). In an animal model of chronic relapsing experimental allergic encephalomyelitis induced by repeated administration to mice of syngenic spinal cord homogenate emulsified in Freund's complete adjuvant, it was demonstrated that PEA alleviated the spasticity found in the hind limbs [55] and also in TMEV-IDD, respectively [56]. These effects of PEA are due in large part to their anti-inflammatory effects. In both CREAE and TMEV-IDD, a reduction in proinflammatory cytokines was observed, accompanied by a decrease in damage and axonal demyelination [56, 57]. On the other hand, in a mouse, significantly reduces the development of clinical signs in the MOG model of EAE accompanied

Figure 2.
Schematic representation of inhibitory effect of palmitoylethanolamide (PEA) on neuroinflammation in neurodegenerative disorders such as hypoxia-ischemia. Neuroinflammation is a common process both for neurodegenerative diseases and for hypoxia-ischemia. This process consists of an inflammatory response carried out by both astrocytes and microglia cells through the release of proinflammatory cytokines, neurotoxic mediators, TNF α , and IL- β . These latter throughout the activation of their own receptors trigger intracellular mechanisms conveying in protein degradation, mitochondria desorganization, axonal dysfunction, and apoptosis, with consequent neurodegeneration at the basis of diseases like Parkinson, Alzheimer, multiple and amyotrophic lateral sclerosis and hypoxia-ischemia. The PEA through the PPAR α activation inhibits the neuroinflammatory process through the increase in the production of anti-inflammatory cytokines, the synthesis of I κ B- α , as well as, favoring the increase of the synthesis of neurotrophic mediators such as: GDNF and BDNF.

by a reduction in transcript expression of the acute-phase protein SAA1, TNF- α , IL-1 β , IFN- γ , and NLRP3 proinflammatory proteins and TLR2, Fpr2, CD137, CD3- γ , TCR- ζ chain, and CB2 receptors [58]. In addition, PEA and PPAR- α (peroxisome proliferator-activated receptor- α agonist) have been effective in reducing symptoms of central neuropathic pain in patients with multiple sclerosis [59].

All these evidences suggest that PEA is a central and promising target in the development of new neuroprotective agents, being PPAR- α the main mechanism by which this neuroprotection would be being exercised. The block or mimic PEA effects were evidenced for the use of PPAR- α agonists and antagonists, supporting this idea [7, 60, 61]. **Figure 2** schematizes how the mechanism of action of PEA is in neurodegenerative diseases such as hypoxia-ischemia.

7. Conclusion

PEA is involved in several processes: lipidic metabolism, proinflammatory cytokine genes transcription, signaling cascade, and proliferation. PEA is expressed in brain, skin, liver, intestine, heart, muscle, kidney, and adipose tissue.

It plays a protective role in several neurological disorders and ischemic brain injury. In vivo assays with PEA treatment showed an improve of the neuroinflammation, reduce astrogliosis, and preserve cognitive functions by PPAR- α activation. In vitro assays, pretreatment with PEA and a flavonoid prevent the degranulation of mast cells and reduce the neurotoxic potential of MC/9 cells in OGD model.

PEA treatment could be a potential alternative therapy than hypothermia. This is because the hypothermia is an unspecific treatment and PEA acts under specific mechanism. However, this sort of therapy is still incipient, to say the least, and further investigations should pursue on focusing in the main downstream effectors involved in HI.

Author details

Lucas D. Udovin^{1*}, Andrea Aguilar¹, Tamara Kobiec², María I. Herrera², Santiago Perez Lloret¹, Nicolás Toro Urrego¹ and Rodolfo A. Kölliker Frers^{1,3}

¹ Institute of Cardiological Research, University of Buenos Aires, National Research Council (ININCA-UBA-CONICET), Buenos Aires, Argentina

² Centro de Investigaciones en Psicología y Psicopedagogía (CIPP), Pontificia Universidad Católica Argentina, Buenos Aires, Argentina

³ Hospital Ramos Mejía, Buenos Aires, Argentina

*Address all correspondence to: lucas2304@hotmail.com

IntechOpen

© 2020 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] Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson N-O, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology*. 2007;**152**(7):1092-1101
- [2] Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, et al. The nuclear receptor peroxisome proliferator-activated receptor- α mediates the anti-inflammatory actions of palmitoylethanolamide. *Molecular Pharmacology*. 2005;**67**(1):15 LP-19 LP
- [3] Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, Kodaka T, et al. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *The Journal of Biological Chemistry*. 2000;**275**(1):605-612
- [4] Lauckner JE, Jensen JB, Chen H-Y, Lu H-C, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;**105**(7):2699-2704
- [5] Henstridge DC, Febbraio MA. Adiponectin sparks an interest in calcium. *Cell Metabolism*. 2010;**11**:447-449
- [6] Godlewski G, Offertáler L, Wagner JA, Kunos G. Receptors for acylethanolamides—GPR55 and GPR119. *Prostaglandins & Other Lipid Mediators*. 2010;**89**:105-111
- [7] LoVerme J, La Rana G, Russo R, Calignano A, Piomelli D. The search for the palmitoylethanolamide receptor. *Life Sciences*. 2005;**77**(14):1685-1698
- [8] Yang H, Zhou J, Lehmann C. GPR55—a putative “type 3” cannabinoid receptor in inflammation. *Journal of Basic and Clinical Physiology and Pharmacology*. 2016;**27**(3):297-302
- [9] Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature*. 1997;**389**(6653):816-824. DOI: 10.1038/39807
- [10] Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*. 2006;**139**(4):1405-1415
- [11] Marzo V, Starowicz K, Cristino L. TRPV1 receptors in the central nervous system: Potential for previously unforeseen therapeutic applications. *Current Pharmaceutical Design*. 2008;**14**(1):42-54
- [12] Petrosino S, Iuvone T, Di Marzo V. N-palmitoyl-ethanolamine: Biochemistry and new therapeutic opportunities. *Biochimie*. 2010;**92**(6):724-727
- [13] Edwards J. TRPV1 in the central nervous system: Synaptic plasticity, function, and pharmacological implications. *Progress in Drug Research*. 2014;**68**:77-104
- [14] Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazarrella E, et al. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: Potential for the

- treatment of neuronal hyperexcitability. ACS Chemical Neuroscience. 2014;**5**(11):1131-1141. DOI: 10.1021/cn5000524
- [15] Cortright DW, Szallasi A. Biochemical pharmacology of the vanilloid receptor TRPV1: An update. European Journal of Biochemistry. 2004;**271**:1814-1819
- [16] 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
- [17] Nagy I, Friston D, Valente JS, Torres Perez JV, Andreou AP. Pharmacology of the capsaicin receptor, transient receptor potential vanilloid type-1 ion channel. Progress in Drug Research. 2014;**68**:39-76
- [18] Portavella M, Rodriguez-Espinosa N, Galeano P, Blanco E, Romero JI, Holubiec MI, et al. Oleoylethanolamide and palmitoylethanolamide protect cultured cortical neurons against hypoxia. Cannabis Cannabinoid Research. 2018;**3**(1):171-178
- [19] Petrosino S, Puigdemont A, Della Valle MF, Fusco M, Verde R, Allara M, et al. Adelmidrol increases the endogenous concentrations of palmitoylethanolamide in canine keratinocytes and down-regulates an inflammatory reaction in an in vitro model of contact allergic dermatitis. Veterinary Journal. 2016;**207**:85-91
- [20] Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990;**346**(6284):561-564
- [21] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993;**365**(6441):61-65
- [22] Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacology & Therapeutics. 1997;**74**(2):129-180
- [23] Turu G, Hunyady L. Signal transduction of the CB1 cannabinoid receptor. Journal of Molecular Endocrinology. 2010;**44**(2):75-85
- [24] Gomez del Pulgar T, Velasco G, Guzman M. The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. The Biochemical Journal. 2000;**347**(Pt 2):369-373
- [25] Sanchez MG, Ruiz-Llorente L, Sanchez AM, Diaz-Laviada I. Activation of phosphoinositide 3-kinase/ PKB pathway by CB(1) and CB(2) cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. Cellular Signalling. 2003;**15**(9):851-859
- [26] Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 2010;**58**(9):1017-1030
- [27] Izzo AA. Cannabinoids and intestinal motility: Welcome to CB2 receptors. British Journal of Pharmacology. 2004;**142**(8):1201-1202
- [28] Pertwee RG. GPR55: A new member of the cannabinoid receptor clan? British Journal of Pharmacology. 2007;**152**(7):984-986
- [29] Campora L, Miragliotta V, Ricci E, Cristino L, Di Marzo V, Albanese F, et al. Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. American Journal of Veterinary Research. 2012;**73**(7):988-995
- [30] Basu S, Dittel BN. Unraveling the complexities of cannabinoid receptor 2

- (CB2) immune regulation in health and disease. *Immunologic Research*. 2011;**51**(1):26-38
- [31] Demuth DG, Molleman A. Cannabinoid signalling. *Life Sciences*. 2006;**78**(6):549-563
- [32] Di Marzo V, Melck D, Orlando P, Bisogno T, Zagoory O, Bifulco M, et al. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. *The Biochemical Journal*. 2001;**358**(Pt 1):249-255
- [33] de Groot H, Rauven U. Ischemia-reperfusion injury: Processes in pathogenetic networks: A review. *Transplantation Proceedings*. 2007;**39**(2):481-484
- [34] Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. *International Review of Cell and Molecular Biology*. 2012;**298**:229-317
- [35] Dugan LL, Choi DW. Hypoxia-ischemia and brain infarction. In: Siegel GJ, Agranoff BW, Albers RW, et al., editors. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. 6th ed. Lippincott-Raven; 1999
- [36] Johnston MV, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic-ischemic injury in the developing brain. *Pediatric Research*. 2001;**49**(6):735-741
- [37] Vannucci SJ, Willing LB, Goto S, Alkayed NJ, Brucklacher RM, Wood TL, et al. Experimental stroke in the female diabetic, db/db, mouse. *Journal of Cerebral Blood Flow and Metabolism*. 2001;**21**(1):52-60
- [38] Uria-Avellanal C, Robertson NJ. Na(+)/H(+) exchangers and intracellular pH in perinatal brain injury. *Translational Stroke Research*. 2014;**5**(1):79-98
- [39] Wang C, Jie C, Dai X. Possible roles of astrocytes in estrogen neuroprotection during cerebral ischemia. *Reviews in the Neurosciences*. 2014;**25**(2):255-268
- [40] Dietrich WD. Neurobiology of stroke. *International Review of Neurobiology*. 1998;**42**:55-101
- [41] Liu L, Yenari MA. Clinical application of therapeutic hypothermia in stroke. *Neurological Research*. 2009;**31**(4):331-335
- [42] Ross OA, Worrall BB, Meschia JF. Advancing stroke therapeutics through genetic understanding. *Current Drug Targets*. 2007;**8**(7):850-859
- [43] Holubiec MI, Romero JI, Suarez J, Portavella M, Fernandez-Espejo E, Blanco E, et al. Palmitoylethanolamide prevents neuroinflammation, reduces astrogliosis and preserves recognition and spatial memory following induction of neonatal anoxia-ischemia. *Psychopharmacology*. 2018;**235**(10):2929-2945
- [44] Sun Y, Alexander SPH, Garle MJ, Gibson CL, Hewitt K, Murphy SP, et al. Cannabinoid activation of PPAR alpha; a novel neuroprotective mechanism. *British Journal of Pharmacology*. 2007;**152**(5):734-743
- [45] Schomacher M, Muller HD, Sommer C, Schwab S, Schabitz W-R. Endocannabinoids mediate neuroprotection after transient focal cerebral ischemia. *Brain Research*. 2008;**1240**:213-220
- [46] Franklin A, Parmentier-Batteur S, Walter L, Greenberg DA, Stella N. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *The Journal of Neuroscience*. 2003;**23**(21):7767-7775

- [47] Caltagirone C, Cisari C, Schievano C, Di Paola R, Cordaro M, Bruschetta G, et al. Co-ultramicrosized palmitoylethanolamide/luteolin in the treatment of cerebral ischemia: From rodent to man. *Translational Stroke Research*. 2016;7(1):54-69
- [48] Ahmad A, Genovese T, Impellizzeri D, Crupi R, Velardi E, Marino A, et al. Reduction of ischemic brain injury by administration of palmitoylethanolamide after transient middle cerebral artery occlusion in rats. *Brain Research*. 2012;1477:45-58
- [49] Herrera MI, Udovin LD, Toro-Urrego N, Kusnier CF, Luaces JP, Capani F. Palmitoylethanolamide ameliorates hippocampal damage and behavioral dysfunction after perinatal asphyxia in the immature rat brain. *Frontiers in Neuroscience*. 2018;12:145
- [50] Parrella E, Porrini V, Iorio R, Benarese M, Lanzillotta A, Mota M, et al. PEA and luteolin synergistically reduce mast cell-mediated toxicity and elicit neuroprotection in cell-based models of brain ischemia. *Brain Research*. 2016;1648(Pt A):409-417
- [51] D'Agostino G, Russo R, Avagliano C, Cristiano C, Meli R, Calignano A. Palmitoylethanolamide protects against the amyloid-beta25-35-induced learning and memory impairment in mice, an experimental model of Alzheimer disease. *Neuropsychopharmacology*. 2012;37(7):1784-1792
- [52] Scuderi C, Stecca C, Valenza M, Ratano P, Bronzuoli MR, Bartoli S, et al. Palmitoylethanolamide controls reactive gliosis and exerts neuroprotective functions in a rat model of Alzheimer's disease. *Cell Death & Disease*. 2014;5:e1419
- [53] Esposito E, Impellizzeri D, Mazzone E, Paterniti I, Cuzzocrea S. Neuroprotective activities of palmitoylethanolamide in an animal model of Parkinson's disease. *PLoS One*. 2012;7(8):e41880
- [54] Avagliano C, Russo R, De Caro C, Cristiano C, La Rana G, Piegari G, et al. Palmitoylethanolamide protects mice against 6-OHDA-induced neurotoxicity and endoplasmic reticulum stress: In vivo and in vitro evidence. *Pharmacological Research*. 2016;113(Pt A):276-289
- [55] Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Makriyannis A, et al. Endocannabinoids control spasticity in a multiple sclerosis model. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2001;15(2):300-302
- [56] Loria F, Petrosino S, Mestre L, Spagnolo A, Correa F, Hernangomez M, et al. Study of the regulation of the endocannabinoid system in a virus model of multiple sclerosis reveals a therapeutic effect of palmitoylethanolamide. *The European Journal of Neuroscience*. 2008;28(4):633-641
- [57] Rahimi A, Faizi M, Talebi F, Noorbakhsh F, Kahrizi F, Naderi N. Interaction between the protective effects of cannabidiol and palmitoylethanolamide in experimental model of multiple sclerosis in C57BL/6 mice. *Neuroscience*. 2015;290:279-287
- [58] Contarini G, Franceschini D, Facci L, Barbierato M, Giusti P, Zusso M. A co-ultramicrosized palmitoylethanolamide/luteolin composite mitigates clinical score and disease-relevant molecular markers in a mouse model of experimental autoimmune encephalomyelitis. *Journal of Neuroinflammation*. 2019;16(1):126
- [59] Kopsky DJ, Hesselink JMK. Multimodal stepped care approach with acupuncture and PPAR-alpha agonist

palmitoylethanolamide in the treatment of a patient with multiple sclerosis and central neuropathic pain. *Acupuncture in Medicine*. 2012;**30**(1):53-55

[60] Sheu MY, Fowler AJ, Kao J, Schmuth M, Schoonjans K, Auwerx J, et al. Topical peroxisome proliferator activated receptor- α activators reduce inflammation in irritant and allergic contact dermatitis models. *The Journal of Investigative Dermatology*. 2002;**118**(1):94-101

[61] Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, et al. The nuclear receptor peroxisome proliferator-activated receptor- α mediates the anti-inflammatory actions of palmitoylethanolamide. *Molecular Pharmacology*. 2005;**67**(1):15-19