Pharmacological Research 113 (2016) 276–289

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Regular article

Palmitoylethanolamide protects mice against 6-OHDA-induced neurotoxicity and endoplasmic reticulum stress: In vivo and in vitro evidence

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ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 5 September 2016
Accepted 6 September 2016
Available online 8 September 2016

Keywords:
Palmitoylethanolamide
Endoplasmic reticulum stress
Oxidative stress
PPAR-α
Apoptosis
Parkinson's disease

ABSTRACT

Several pathogenetic factors have been involved in the onset and progression of Parkinson's disease (PD), including inflammation, oxidative stress, unfolded protein accumulation, and apoptosis. Palmitoylethanolamide (PEA), an endogenous N-acylethanolamine, has been shown to be a neuroprotective and anti-inflammatory molecule, acting as a peroxisome proliferator activated receptor (PPAR)-α agonist.

In this study we investigated the effects of PEA on behavioral alterations and the underlying pathogenic mechanisms in the 6-hydroxydopamine (6-OHDA)-induced model of PD in male mice. Additionally, we showed the involvement of PPAR-α in PEA protective effect on SH-SY5Y neuroblastoma against 6-OHDA damage.

Here, we report that PEA (3–30 mg/kg/day s.c.) improved behavioral impairments induced by unilateral intrastriatal injection of 6-OHDA. This effect was accompanied by a significant increase in tyrosine hydroxylase expression at striatal level, indicating PEA preserving effect on dopaminergic neurons. Moreover, we found a reduction in the expression of pro-inflammatory enzymes, i.e. inducible nitric oxide synthase and cyclooxygenase-2, a modulation between pro- and anti-apoptotic markers, suggestive of PEA capability in controlling neuroinflammation and cell death. Interestingly, PEA also showed protective scavenging effect, through superoxide dismutase induction, and dampened unfolding protein response, interfering on glucose-regulated protein 78 expression and PERK-eIF2α pathway. Similar data were found in in vitro studies, where PEA treatment was found to rescue SH-SY5Y neuroblastoma cells from 6-OHDA-induced damage and death, partly by inhibiting endoplasmic reticulum stress detrimental response. Therefore, PEA, counteracting the pathogenetic aspects involved in the development of PD, showed its therapeutic potential, possibly integrating current treatments correcting dopaminergic deficits and motor dysfunction.

Abbreviations: PEA, palmitoylethanolamide; PPAR, peroxisome proliferator activated receptor; PD, Parkinson's disease; DA, dopamine; ER, endoplasmic reticulum; UPR, unfolded protein response; eIF-2α, elongation initiation factor-2α; 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TH, tyrosine hydroxylase; BiP/Grp78, Binding immunoglobulin Protein/Glucose-Regulated Protein 78; SOD, superoxide dismutase; ROS, reactive oxygen species; RNS, reactive nitrogen species; iNOS, inducible nitric oxide synthase; COX, cicloxygenase; NF-κB, nuclear factor kappa B; Bcl-2, B-cell lymphoma 2; BAX, Bcl-2-associated X protein; AEA, anandamide; NAAA, N-acylethanolamine-hydrolysing acid amidase.

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http://dx.doi.org/10.1016/j.phrs.2016.09.004
1043-6618/

1. Introduction

Parkinson's disease (PD) is a progressive, neurodegenerative disorder that is associated with the loss of dopamine-producing neurons in the *substantia nigra pars compacta*,causing dopamine (DA) depletion in the *striatum* where these neurons project.Indeed, DA relays messages between the *substantia nigra* and other parts of the brain, controlling voluntary movements of the body. Therefore, PD is characterized by motor symptoms, such as resting tremor, postural imbalance, slowness of movement and rigidity.

The main mechanisms involved in dopaminergic neuronal vulnerability in PD include oxidative stress, impaired calcium homeostasis, mitochondrial dysfunction, alteredendoplasmic reticulum (ER)-to-Golgi trafficking, and altered mitophagy and proteasomefunction, among other events [1]. Many of these alterations (e.g. perturbed calcium homeostasis, mitochondrial dysfunction, and free radical exposure) can adversely affect protein folding and result in the accumulation of misfolded or unfolded proteins in the ER lumen, causing ER stress [2]. Indeed, the accumulation of misfolded proteins in the brain has been shown in several neurodegenerative disorders, including Alzheimer's disease, amyotrophic lateral sclerosis, in addition to PD, introducing a new classification of these diseases as protein misfolding disorders [3]. In the attempt to deal with ER stress, ER responds by triggering specific signaling pathways including the unfolded protein response (UPR), in order to reduce the amount of newly synthesized proteins translocated into the ER lumen, and increase the degradation of misfolded proteins and increase the protein folding capacity [4]. UPR is characterized by the up-regulation of genes encoding ER chaperon proteins, such as immunoglobulin binding protein (BiP), also known as glucose-regulated protein 78 (Grp78), and glucose-regulated protein 94 (Grp94), which enhance protein folding activity and prevent protein aggregation in the ER [5]. Moreover, UPR induces translational suppression in order to reduce the load of protein synthesis through eukaryotic initiation factor (eIF) 2α phosphorylation, resulting in the prevention of further accumulation of unfolded proteins. However, if the stress is prolonged, these pro-survival mechanisms fail to rescue the cells, then the balance between pro-apoptotic and anti-apoptotic proteins, belonging to pro-apoptotic Bcl-2 family members, induce the transition from a protective to an apoptotic UPR response [6,7].Therefore, the UPR, integrating information about protein misfolding at the ER, regulates cell fate through a variety of complementary mechanisms. This cellular distress has been recognized as an early component of PD pathogenesis and a potential pathway contributing to dopaminergic neuron loss [8]. Among neurotoxins used to model PD, 6-hydroxydopamine (6-OHDA) mimics the selective dopaminergic neurodegeneration through the induction of ER stress and enhancement of oxidative stress, and replicates most of the neuropathological hallmarks of PD [9,10].

Peroxisome proliferator activated receptor (PPAR)-α has been suggested as molecular target for slowing several neurodegenerative disorders, including PD [11,12]; in particular, fenofibrate, but not bezafibrate, has been demonstrated to prevent dopaminergic neuronal loss in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD in mice [13]. The expression of PPAR-α in dopamine neurons of *substantia nigra* and spiny neurons of dorsal striatum [14,15] strongly indicated the involvement of a PPAR-α-mediated mechanism. More recently, Esposito et al. [12] showed the protective role of palmitoylethanolamide (PEA), an endogenous PPAR-α agonist, against MPTP-induced neurotoxicity, through a PPAR-α mediated mechanism, since PEA failed to exert its effect in PPAR-α knock-out mice. Chemically, PEA belongs to the family of acylethanolamides (AEs) that include the endocannabinoid anandamide and the anorectic mediator oleoylethanolamide. Anandamide (AEA), oleoylethanolamide and PEA share anabolic and catabolic enzymes involved in their biosynthesis and degradation [16,17], a part from N-acylethanolamine acid amidase (NAAA), that has been identified as a key specific enzyme involved in PEA degradation during inflammation [18]. Several lines of evidence indicate the existence of a cross talk between the endocannabinoid and dopaminergic systems in brain areas regulating motor function, in fact CB_1 and DA receptors co-localized in striatal neurons, endocannabinoids influence the firing activity of dopaminergic neurons and DA release in vivo and lastly the stimulation of DA receptors increases AEA levels in the basal ganglia [19,20].

It is now established that PEA is biosynthesized to maintain cellular homeostasis when this is challenged by external stressors provoking inflammation, neuronal damage and pain [21]. In the past, it was proposed the idea that PEA was a cannabinoid receptor (CB_2) agonist [22], conversely, it has been shown that PEA had no effect in PPAR-α knock-out mice [23]. To date, it is widely recognized that the main PEA pharmacological effects are mediated by activation of PPAR-α [24]. Indeed, the discovery of PPAR-α in distinct areas of the brain, has opened a new scenario to explore the possible activity of these AEs in the central nervous system [14]. Based on the aforementioned evidence, we sought to investigate the effects of PEA treatment (3–30 mg/kg) in an in vivo mouse model of PD induced by the striatal injection of 6-OHDA. We examined the protective effect of PEA on behavioral alterations (i.e., locomotor tests), and biochemical modifications leading to oxidative stress, apoptosis and ER stress. Additionally, we evaluated the protective effects of PEA against neuronal damage of 6-OHDA, using SH-SY5Y neuroblastoma, and mechanistically evaluated PPAR-α involvement in PEA reverting effect on oxidative stress, and apoptotic and UPR pathways.

2. Materials and methods

2.1. Chemicals

PEA, GW7647 (2-[[4-[2-[[(Cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl] thio]-2- methylpropanoic acid), a PPAR-α agonist, and GW6471 (*N*-((2*S*)-2-(((1*Z*)-1-Methyl-3-*oxo*-3-(4-(trifluoromethyl) phenyl) prop-1-enyl) amino)-3-(4-(2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy)phenyl)propyl) propanamide), a PPAR-α antagonist, were purchased from Tocris (Bristol, UK). 6-OHDA, apomorphine and retinoic acid (RA) were purchased from Sigma- Aldrich (Milan, Italy).

Dulbecco's modified Eagle's medium-Ham F-12 (DMEM-F12), Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS) and cell culture supplements were purchased from Cambrex Bio Science Verviers (B-800; Verviers, Belgium).

2.2. Animals

Ten week old male Swiss CD1 mice (20–25 g) were purchased from Harlan (Udine, Italy). They were housed in cages in a room kept at 22 ± 1 °C on a 12/12 h light/dark cycle. The animals were acclimated to their environment for 1 week, and had *ad libitum* access to tap water and standard rodent chow. All procedures involving animals were carried out in accordance with the Institutional Guidelines and complied with the Italian D.L. and associated guidelines of the European Communities Council Directive (2010). The procedures reported here were approved by the Institutional Committee on the Ethics of Animal Experiments (CSV) of the University of Naples "Federico II" and by the Ministero della Salute under protocol no. 2014-0084607.