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Neuroscience Letters 404 (2006) 342–346
Neuroscience Letters
www.elsevier.com/locate/neulet

CB_1 receptor selective activation inhibits β-amyloid-induced iNOS protein expression in C6 cells and subsequently blunts tau protein hyperphosphorylation in co-cultured neurons

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Received 16 March 2006; received in revised form 26 May 2006; accepted 5 June 2006

Abstract

Among the wide range of neuro-inflammatory signalling molecules released by β-amyloid-stimulated astroglial cells, nitric oxide (NO) plays a fundamental role in AD aethiopathogenesis since it directly promotes neuronal tau protein hyperphosphorylation leading to neurofibrillary tangle formation. Synthetic cannabinoids (CBs), via a selective CB1 receptor activation, negatively modulates both iNOS protein expression and NO production induced by pro-inflammatory stimuli. In this study we investigated the role of both the non-selective WIN 55,212-2 and the selective CB1 receptor agonist, ACEA, on: (i) NO production, (ii) iNOS protein expression in (1–42) β-amyloid peptide (Aβ)-stimulated C6 rat glioma cells and (iii) tau protein hyperphosphorylation in co-cultured differentiated PC12 neurons. Our results demonstrated that synthetic CBs, by a selective CB1 effect, down-regulate iNOS protein expression and NO production in Aβ-stimulated C6 cells. This effect leads, in turn, to a significant and concentration-dependent inhibition of NO-dependent tau protein hyperphosphorylation in co-cultured PC12 neurons. The results of the present study extend our knowledge about the neuroprotective actions of synthetic CBs on Aβ-dependent neurotoxicity in vitro. Furthermore, our study allows us to identify, in the CB1-mediated inhibition of astroglial-derived NO, a new potential target to blunt tau hyperphosphorylation and the consequent related tauopathy in AD.

Keywords: Alzheimer's disease; Tau protein hyperphosphorylation; Nitric oxide (NO); Cannabinoids; CB1 receptors

Neurofibrillary tangles (NFTs), resulting from an abnormal phosphorylation process of microtubule-associated tau proteins, represent a key pathological hallmark of Alzheimer's brain [8]. The observation that NFTs are co-localised with a broad variety of astrocyte-derived inflammatory proteins (including complement proteins, acute-phase proteins, pro-inflammatory cytokines) and clusters of activated microglia, highlighted the role of reactive gliosis in mediating the early pathogenic events triggering to Alzheimer's disease (AD) [22]. In vitro and in vivo studies demonstrated that, an aberrant disposition of β-amyloid peptide (Aβ) promotes a marked neuroinflammatory scenario, accounting for the synthesis of different cytokines and pro-inflammatory mediators. Those ones may act as paracrine effectors in AD-related neuropathogenesis [13]. Among the wide range of the different pro-inflammatory proteins produced in response to Aβ, inducible nitric oxide synthase (iNOS) and its enzymatic product, nitric oxide (NO), are considered important neurotoxic effectors during AD. NO is produced by the oxidation of a terminal guanidine nitrogen of L-arginine, a reaction catalysed by the enzime NO synthase (NOS) [15]. NO generated by astroglial cells induces, in a paracrine way, tau protein hyperphosphorylation in co-cultured neurons [21]. Thus, the pharmacological inhibition of NO deriving from activated glial cells has been identified as a novel strategy to inhibit tau protein hyperphosphorylation and NFTs formation in AD [17].

Endogenous and exogenous cannabinoids (CBs) have shown to possess neuroprotective effects in different studies [18,19,12] by binding to CB1 receptors. Moreover, in addition to direct neu-

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doi:10.1016/j.neulet.2006.06.012

roprotective effect on neurons, CBs have also been demonstrated to induce anti-inflammatory actions, that may involve NO signalling. CB1 selective activation has been previously reported to down-regulate iNOS protein expression and NO release from cultured C6 rat glioma cells challenged with both LPS and HIV-1 Tat protein [6,7]. On the basis of this background, in the present study we investigated the role of CBs on NO production and iNOS protein expression in C6 rat glioma cells exposed to Aβ and the effect on tau protein hyperphosphorylation in co-cultured, differentiated, PC12 neurons.

All the materials for cell culture were purchased from Biowittaker (Caravaggio, BG, Italy). Foetal calf serum and horse serum were from Hyclone (Logan, UT, USA). Human-amyloid peptide (fragment 1–42) (Aβ), the non-elective CB1/CB2 agonist WIN 55,212-2 ((R-(+)-[2,3-dihydro-5-methyl-3-(4-morpholynilmethyl)pyrrolo[1,2,3-de]-1,4-benzoazin-6-yl]-1-naphtalenilmethanone mesilate), selective CB1 agonist ACEA (arachidonoyl-2′-chloroethylamide/*N*-(2-chloroethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide), JWH015 (2-methyl-1-propyl-1H-indol-3-yl)-1 naphtalenylmethanone) were from Tocris (Hung Road, Bristol, UK), the selective CB1 and CB2 receptor antagonists, respectively, SR141716A and SR144528 were a gift from Dr. Madaleine Moss (Sanofi Research). Anti-iNOS antibody was from BD-Bioscience (Milan, Italy), anti-tau antibody was from Neomarker (Freemont, CA, USA). Anti-mouse IgG was from Dako (Glostrup, Denmark).

C6 rat glioma cells (American Type Tissue Collection CCL-107) were cultured in 10 cm Petri dishes in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% Foetal Bovine Serum (FBS), 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C in 5% CO_2/95% air. Confluent cells were washed three times with Phosphate Buffer Saline (PBS), detached with tripsyn/EDTA, plated in 6 multiwell plates and left to adhere for 24 h at 37 °C. Thereafter, cell medium was replaced with fresh medium, confluent C6 cells were treated with β-amyloid peptide (Aβ) (1–42) (1 μg/mL) for 24 h in the presence or absence of the following substances given 15 min before Aβ: WIN (10^{-8} to 10^{-6} M), ACEA (10^{-9} to 10^{-6} M), JWH015 (10^{-6} to 10^{-6} M). In some experiments the effect of SR141716A (10^{-6} M) or SR144528 (10^{-6} M), in presence of ACEA (10^{-6} M) was also tested.

Rat pheocromocytoma PC12 cells (American Tissue Type Collection CRL-1721) were cultured in 10 cm Petri dishes in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% Foetal Bovine Serum (FBS), 15% Horse Serum (HS), 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C in 5% CO_2/95% air, and differentiated with an appropriate concentration of retinoic acid [4]. PC12 cells were co-cultured with Aβ-treated C6 cells.

In co-culture experiments, confluent PC12 cells were seeded in sterile Falcon inserts at the density of 2.5×10^5 cells/well. In parallel, C6 cells (1×10^6 cells/well) were treated as above described. Then, the C6 cell supernatant was removed and cells were extensively washed to remove any residual of Aβ (1–42) peptide and tested drugs and C6 cells were supplemented with fresh medium and PC12 cells were inserted in the 6-multiwell plate. PC12 and C6 cells were then co-cultured for a further 24 h at 37 °C After this time, the inserts were removed and PC12 were gently detached and lysed as described [4].

Production of NO was assayed by measuring the amount of nitrite in the culture medium of C6 cells treated as above described by using a spectrophotometric assay based on Griess reaction [3].

The expression of iNOS protein, in C6 cells, and tau protein, in PC12 cells, was evaluated by Western blot analysis. Briefly, harvested cells (1×10^6) were washed two times with ice-cold PBS and centrifuged at $180 \times g$ for 10 min at 4 °C. The cell pellet was resuspended in 100 μl of ice-cold hypotonic lysis buffer (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM phenylmethylsulphonylfluoride, 1.5 μg/ml soybean trypsin inhibitor, 7 μg/ml pepstatin A, 5 μg/ml leupeptin, 0.1 mM benzamidine, 0.5 mM DTT) and incubated on ice for 15 min. The cells were lysed by rapid passage through a syringe needle for five to six times and the cytoplasmic fraction was then obtained by centrifugation for 1 min at $13{,}000 \times g$. Cytosolic fraction proteins were mixed with non-reducing gel loading buffer (50 mM Tris/10% SDS/10% glycerol/10% /2 mg of bromophenol per ml) in a ratio of 1:1, boiled for 3 min and centrifuged at $10{,}000 \times g$ for 10 min. Protein concentration was determined and equivalent amounts (100 μg) of each sample were electrophoresized in a 12% discontinuous polyacrylamide minigel. The proteins were transferred onto nitro-cellulose membranes, that were saturated by incubation at 4 °C overnight with 10% non-fat dry milk in PBS and then incubated respectively with (1:2000, v/v) anti-mouse monoclonal iNOS, (1:250, v/v) anti-mouse monoclonal tau and (1:1000, v/v) anti-mouse monoclonal anti tubulin antibodies. The membranes were washed three times with 1% Triton 100-X in PBS and then incubated with anti-mouse immunoglobulins coupled to peroxidase (1:2000, v/v). The immunocomplexes were visualised by the ECL chemiluminescence method (Amersham, Buckinghamshire, UK). Subsequently, the relative expression of iNOS and tau protein was quantified by densitometric scanning of the X-ray films with a GS 700 Imaging Densitometer (Bio-Rad, Hercules, CA, USA) and a computer programme (Molecular Analyst, IBM).

Results were expressed as mean ± S.E.M. of *n* experiments. Statistical analysis was performed using analysis of variance (ANOVA) and multiple comparisons were performed by Bonferroni's test with $P < 0.05$ considered significant.

Stimulation of C6 cells with Aβ (1–42) (1 μg/ml) for 24 h caused a significant increase of nitrite production (as stable metabolites of NO) compared to un-stimulated cells. WIN 212-2 (10^{-8} to 10^{-6} M) and, at an higher extent ACEA (10^{-9} to 10^{-6} M), given to the cells 15 min before Aβ (1–42), significantly and concentration-dependently inhibited nitrite production in the cell supernatant. On the contrary, JWH-015 (10^{-9} to 10^{-6} M) was un-effective. The effect of ACEA (10^{-6} M) on NO productionwas reversed by the selective CB1 receptor antagonist, SR141716A (10^{-6} M) but not by the selective CB2 receptor antagonist SR144528 (10^{-6} M) (Fig. 1A). In parallel to nitrite production, stimulation of C6 cells with Aβ (1–42) (μg/mL) caused a significant increase of iNOS protein expression compared to un-stimulated cells. ACEA (10^{-8} to 10^{-6} M), given

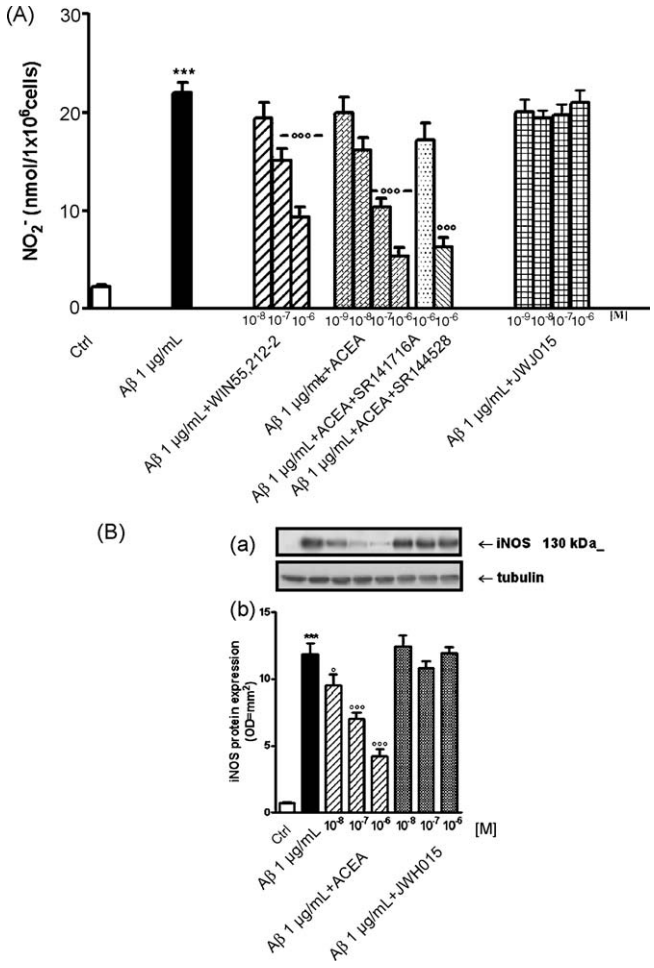

Fig. 1. (A) Effect of WIN 55,212-2 (10^{-8} to 10^{-6} M), ACEA (10^{-9} to 10^{-6} M), JWH015 (10^{-9} to 10^{-6} M), SR141716A (10^{-6} M) and SR144528 (10^{-6} M) on Aβ-induced nitrite production in C6 cells at 24 h. NO production was determined by measuring nitrite (NO_2^-) accumulation in the culture medium. Each bar shows the mean ± S.E.M. of $n = 6$ experiments. $^{***}P < 0.001$ vs. control; $^{ooo}P < 0.001$ vs. Aβ. (B) Western blot analysis showing the effect of ACEA (10^{-8} to 10^{-6} M) and JWH015 (10^{-8} to 10^{-6} M) on Aβ-induced iNOS protein expression at 24 h in C6 cells. Panel (a), showing iNOS protein expression in cell homogenates, is representative of $n = 3$ separated experiments; (b) densitometric analysis of corresponding bands (optical density); each bar shows the mean ± S.E.M. of $n = 3$ experiments. $^{***}P < 0.001$ vs. control.

15 min before Aβ (1–42), concentration dependently reduced iNOS protein expression, as shown by immunoblotting analysis, while JWH-015 (10^{-8} to 10^{-6} M) was without effect (Fig. 1B).

Stimulation of C6 cells with Aβ (1–42) (1 μg/ml) for 24 h lead to a significant increase in hyperphosphorylated tau protein in co-cultured differentiated PC12 neurons. ACEA (10^{-8} to 10^{-6} M), but not JWH-015 (10^{-8} to 10^{-6} M) treatment to Aβ-stimulated C6 cells selectively reduced, in a significant and concentration dependent manner, tau hyperphosphorilatyon in co-cultured PC12 cells (Fig. 2).

Nowadays, it is generally recognized the pivotal role of reactive gliosis in mediating early pathogenic events triggering to neurotoxicity observed in AD. In fact, several in vitro studies demonstrated that cultured astroglial cells secrete high levels of pro-inflammatory cytokines when stimulated with Aβ peptide [11,27], which exert, in turn, toxic effects on neighbouring neurones. The goal of our study was to block the "rebound" toxicity on neurons, by preventing glial cell activation in a model of co-cultures glial cells/neurones which has been extensively used by other authors as a model to study the Aβ neurotoxicity in vitro [20,26]. Among all the compounds having a neuroprotective effect, cannabinoids exert several anti-inflammatory roles, including the inhibition of the pro-inflammatory mediator nitric oxide (NO). Increased amounts of NO, released by activated astrocytes and microglia following Aβ challenge, accelerate neuronal protein nitration [2] and cause a marked increase in tau protein hyperphosphorylation, playing a pivotal role in the progression of AD-related-pathology. For this reason, the blockade of NO production has been postulated to exert beneficial effects in AD [17]. Therefore, we firstly tried to block Aβ-induced NO production in C6 cells by using the non-selective cannabinoid agonist, WIN 55,212-2. Our results show that, WIN 55,212-2 was able to reduce NO production in Aβ-stimulated C6 cells and this effect was due to a selective agonism on CB1 receptors, since the potent and highly selective CB1 receptor agonist, ACEA [9], but not the selective CB2 receptor agonist, JWH015, inhibited NO production in Aβ stimulated C6 cells. Moreover, the ACEA effect was reversed by CB1 selective antagonist SR141716A but not by the selective CB2 antagonist, SR144528. Recent evidences reported that iNOS protein expression is up-regulated in AD brains and its over-expression is recognized to be important in the neuroinflammatory-neurodegenerative process occurring in AD [23]. According to this, in our experiments selectively ACEA, but not JWH015, inhibited iNOS expression in Aβ-stimulated C6 cells, paralleling the effect on NO production.

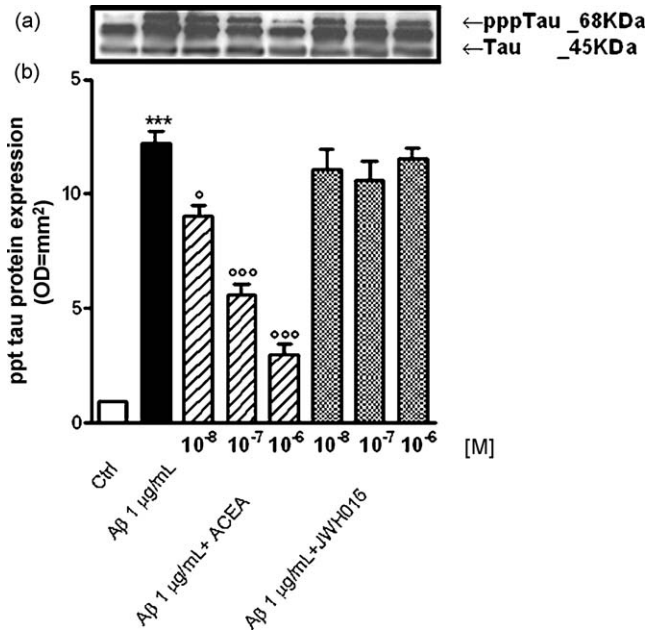

Fig. 2. Western blot analysis showing the effect of ACEA (10^{-8} to 10^{-6} M) and JWH015 (10^{-8} to 10^{-6} M) on tau protein hyperphosphorylation in PC12 cells co-cultured with Aβ-stimulated C6 cells. Panel (a), showing tau and hyperphosphorylated tau (pppTau) protein expression in cell homogenates, is representative of $n = 3$ separated experiments; (b) densitometric analysis of corresponding bands (optical density); each bar in panel B shows the mean ± S.E.M. of $n = 3$ experiments. $^{***}P < 0.001$ vs. control; $^{o}P < 0.01$, $^{ooo}P < 0.001$ vs. Aβ.

Finally, in this study we give evidence that, the CB1-mediated inhibition, via ACEA, of the Aβ-induced NO production occurring in C6 cells leads, in turn, to a significant inhibition of NO-dependent tau protein hyperphosphorylation in co-cultured PC12 neurons.

Several considerations may be taken into account to strengthen the relevance of the here-described results. Indeed, neuroprotective properties of CB1 receptor activation have been previously investigated in different experimental in vitro and in vivo acute neurodegenerative conditions, such as excitotoxic damage [24], ischemia [16] and acute brain injury [18,25]. Moreover, CB1 receptor-mediated neuroprotective effects have been reinforced by different studies showing CB1-knock out animals are more susceptible to neurodegeneration [19,12]. However, in AD, the relevance of CB1-mediated neuroprotection is still under debate in view of different reasons not least considering that in AD brains CB1 positive neuron density is largely decreased in comparison with that observed in healthy subjects. On the contrary, recent evidences show that the cannabinoid CB2 receptors are selectively over-expressed in neuritic plaques-associated glia in AD brain [1], but the role of this overexpression is still unclear thus agreeing with our data which here report the lack of effects of JWH015. In addition, given that CB1 receptors are mainly expressed in neurons segregated by senile plaques, it is possible to speculate that their putative contribution to neuroprotection is consequently very limited [20] as well as it is also possible to assume that, in these conditions, astroglia may constitute the preminent source of CB1 receptors. Since previous studies have provided evidence that astroglial CB1 receptor signalling works as a molecular mechanism leading to NO inhibition in different experimental conditions [6,7], this may suggest that the role of CB1 activation should not be longer restricted to that of a straight defence process operated merely on neuronal cells. In fact, CB1-receptors appears to exert marked anti inflammatory effects on activated astrocytes and microglial cells, resulting in a sharp reduction of several pro-inflammatory mediators during chronic neurodegenerative conditions [6]. This accounts for an effective "rebound protection" exerted on neighbouring neurons. This hypothesis is reinforced by our study, since PC12 cells are not endowed with CB1 receptors, as previously demonstrated by us and accordingly to Molderings et al. [14]. Therefore, the above-described inhibition of tau hyperphosphorylation in PC12 is due to the blockade of NO production through the selective CB1 agonism on C6 cells. Firstly, although in our study CB2 receptor has not been involved in neuroprotection, recent evidences show that the cannabinoid CB2 receptors are selectively over-expressed in neuritic plaques-associated glia in AD brain [1]. This result is in agreement with our recent evidence pointing out that the non-psychoactive natural cannabinoid compound cannabidiol, which acts by non-CB1 mediated mechanism, exerts a combination of neuroprotective effects in Aβ-stimulated PC12 neurones, preventing neurotoxicty, tauopathy and neuroinflammation [10,4,5].

In conclusion, the results of the present study help to extend our knowledge about the neuroprotective actions of synthetic cannabinoids towards Aβ stimulus in vitro, and they identify in the CB1-mediated inhibition of astroglial-deriving NO a further target useful to counteract tau hyperphosphorylation to contrast AD related-tauopathies.

Acknowledgements

Cofin MURST 2004; FIRB 2006.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2006.06.012.

References

[1] C. Benito, E. Nunez, R.M. Tolon, E.J. Carrier, A. Rabano, C.J. Hillard, J. Romero, Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains, J. Neurosci. (23) (2003) 11136–11141.
[2] M. Coma, F.X. Guix, I. Uribesalgo, G. Espuna, M. Sole, D. Andreu, F.J. Munoz, Lack of oestrogen protection in amyloid-mediated endothelial damage due to protein nitrotyrosination, Brain 128 (2005) 1613–1621.
[3] M. Di Rosa, M. Radomski, R. Carnuccio, S. Moncada, Glucocorticoids inhibit the induction of nitric oxide synthase in macrophages, Biochem. Biophys. Res. Commun. 172 (1990) 1246–1252.
[4] G. Esposito, D. De Filippis, R. Carnuccio, A.A. Izzo, T. Iuvone, The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells, J. Mol. Med. 84 (2006) 253–258.
[5] G. Esposito, D. De Filippis, M.C. Maiuri, D. De Stefano, R. Carnuccio, T. Iuvone, Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in β-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-κB involvement, Neurosci. Lett. (2006), Epub ahead of print.
[6] G. Esposito, A.A. Izzo, M. Di Rosa, T. Iuvone, Selective cannabinoid CB1 receptor-mediated inhibition of inducible nitric oxide synthase protein expression in C6 rat glioma cells, J. Neurochem. 78 (2001) 835–841.
[7] G. Esposito, A. Ligresti, A.A. Izzo, T. Bisogno, M. Ruvo, M. Di Rosa, V. Di Marzo, T. Iuvone, The endocannabinoid system protects rat glioma cells against HIV-1 Tat protein-induced cytotoxicity. Mechanism and regulation, J. Biol. Chem. 27 (2002) 50348–50354.
[8] I. Grundke-Iqbal, K. Iqbal, M. Quinlan, Y.C. Tung, M.S. Zaidi, H.M. Wisniewski, Microtubule-associated protein tau. A component of Alzheimer paired helical filaments, J. Biol. Chem. 261 (1986) 6084–6089.
[9] C.J. Hillard, S. Manna, M.J. Greenberg, R. DiCamelli, R.A. Ross, L.A. Stevenson, V. Murphy, R.G. Pertwee, W.B. Campbell, Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1), CJ J. Pharmacol. Exp. Ther. 289 (1999) 1427–1433.
[10] T. Iuvone, G. Esposito, R. Esposito, R. Santamaria, M. Di Rosa, A.A. Izzo, Neuroprotective effect of cannabidiol, a non-psychoactive component from *Cannabis sativa*, on beta-amyloid-induced toxicity in PC12 cells, J. Neurochem. (89) (2004) 134–141.
[11] S.U. Kim, J. de Vellis, Microglia in health and disease, J. Neurosci. Res. 81 (2005) 302–313.
[12] G. Marsicano, S. Goodenough, K. Monory, H. Hermann, M. Eder, A. Cannich, S.C. Azad, M.G. Cascio, S.O. Gutierrez, M. van der Stelt, M.L. Lopez-Rodriguez, E. Casanova, G. Schutz, W. Zieglgansberger, V. Di Marzo, C. Behl, B. Lutz, CB1 cannabinoid receptors and on-demand defense against excitotoxicity, Science 302 (2003) 84–88.
[13] P.L. McGeer, E.G. McGeer, Inflammation, autotoxicity and Alzheimer disease, Neurobiol. Aging 6 (2001) 799–809.
[14] G.J. Molderings, H. Bonisch, R. Hammermann, M. Gothert, M. Bruss, Noradrenaline release-inhibiting receptors on PC12 cells devoid of alpha(2(−)) and CB(1) receptors: similarities to presynaptic imidazoline and edg receptors, Neurochem. Int. (40) (2002) 157–167.

[15] S. Moncada, E.A. Higgs, Endogenous nitric oxide: physiology, pathology and clinical relevance, Eur. J. Clin. Invest. 21 (1991) 361–374.
[16] T. Nagayama, A.D. Sinor, R.P. Simon, J. Chen, S.H. Graham, K. Jin, D.A. Greenberg, Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures, J. Neurosci. 19 (1999) 2987–2995.
[17] C. Nathan, N. Calingasan, J. Nezezon, A. Ding, M.S. Lucia, K. La Perle, M. Fuortes, M. Lin, S. Ehrt, N.S. Kwon, J. Chen, Y. Vodovotz, K. Kipiani, M.F. Beal, Protection from Alzheimer's-like disease in the mouse by genetic ablation of inducible nitric oxide synthase, J. Exp. Med. 202 (2005) 1163–1169.
[18] D. Panikashvili, R. Mechoulam, S.M. Beni, A. Alexandrovich, E. Shohami, CB1 cannabinoid receptors are involved in neuroprotection via NF-kappa B inhibition, J. Cereb. Blood Flow Metab. 25 (2005) 477–484.
[19] S. Parmentier-Batteur, K. Jin, X.O. Mao, L. Xie, D.A. Greenberg, Increased severity of stroke in CB1 cannabinoid receptor knock-out mice, J. Neurosci. 22 (2002) 9771–9775.
[20] B.G. Ramirez, C. Blazquez, T. Gomez del Pulgar, M. Guzman, M.L. de Ceballos, Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation, J. Neurosci. 25 (2005) 1904–1913.
[21] T.E. Saez, M. Pehar, M. Vargas, L. Barbeito, R.B. Maccioni, Astrocytic nitric oxide triggers tau hyperphosphorylation in hippocampal neurons, In Vivo 18 (2004) 275–280.
[22] M.A. Smith, M. Vasak, M. Knipp, R.J. Castellani, G. Perry, Dimethylargininase, a nitric oxide regulatory protein, in Alzheimer disease, Free Radic. Biol. Med. 25 (1998) 898–902.
[23] T. Togo, O. Katsuse, E. Iseki, Nitric oxide pathways in Alzheimer's disease and other neurodegenerative dementias, Neurol. Res. 26 (2004) 563–566.
[24] M. van der Stelt, V. Di Marzo, Cannabinoid receptors and their role in neuroprotection, Neuromol. Med. 7 (2005) 37–50.
[25] M. van der Stelt, W.B. Veldhuis, P.R. Bar, G.A. Veldink, J.F. Vliegenthart, K. Nicolay, Neuroprotection by Delta9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity, J. Neurosci. (21) (2001) 6475–6479.
[26] B. Vincent, J.D. Smith, Astrocytes down-regulate neuronal beta-amyloid precursor protein expression and modify its processing in an apolipoprotein E isoform-specific manner, Eur. J. Neurosci. 14 (2001) 256–266.
[27] D.G. Walker, L.F. Lue, Investigations with cultured human microglia on pathogenic mechanisms of Alzheimer's disease and other neurodegenerative diseases, J. Neurosci. Res. 81 (2005) 81412–81425.