Life Sciences 155 (2016) 123–132

Contents lists available at ScienceDirect

Life Sciences

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

Reversal effect of simvastatin on the decrease in cannabinoid receptor 1 density in 6-hydroxydopamine lesioned rat brains

Nikolce Mackovski [a,b,c,1], Jinchi Liao [a,1], Ruihui Weng [a,1], Xiaobo Wei [a], Rui Wang [a], Zhaoyu Chen [a], Xu Liu [a], Yinghua Yu [b,c], Barbara J. Meyer [b,c], Ying Xia [d], Chao Deng [b,c], Xu-Feng Huang [b,c,*], Qing Wang [a,b,**]

[a] Department of Neurology, The Third Affiliated Hospital of Sun-Sen University, Tianhe Road 600, Guangzhou, Guangdong 510630, People's Republic of China
[b] Centre of Translational Neurosciences, School of Medicine, University of Wollongong, NSW, 2522, Australia
[c] Illawarra Health and Medical Research Institute, NSW 2522, Australia
[d] Department of Neurosurgery, The University of Texas Medical School at Houston, TX 77030, USA

ARTICLE INFO

Article history:
Received 10 October 2015
Received in revised form 25 April 2016
Accepted 1 May 2016
Available online 4 May 2016

Keywords:
Parkinson's disease
Cannabinoid 1 receptor
6-hydroxydopamine
Simvastatin
[^{3}H] SR141716A

ABSTRACT

Aims: Cannabinoid 1(CB1) receptors are closely correlated to the dopaminergic system and involved in cognitive function. Since statins have been used to regulate the progression of Parkinson's disease (PD) via its anti-inflammation and neuroprotective effects, we asked if statins affect the CB1 receptors in the 6-hydroxydopamine (6-OHDA) lesioned rat.

Methods: The PD rat model was established by injecting 6-OHDA into the unilateral medial forebrain bundle; while rats were orally pre-treated with simvastatin (1 or 10 mg/kg/day), or saline for 5 days before surgery, and the same treatments for each group were continued for 3 weeks post-surgery. [^{3}H] SR141716A binding autoradiography was adopted to investigate the alterations in CB1 receptor density in the brains.

Findings: The 6-OHDA induced a remarkable downregulation of CB1 receptor density in the prefrontal cortex, caudate putamen, nucleus accumbens, cingulate cortex, hippocampus, and substantia nigra; while simvastatin (10 mg/kg/day) significantly ameliorated this downregulation in those regions. Furthermore, simvastatin (1 mg/kg/day) reversed the 6-OHDA-induced downregulation of CB1 receptors in the substantia nigra and hippocampus. Simvastatin showed minimal changes in [^{3}H] SR141716A binding in the examined regions in sham rats, but did reveal a significant down-regulation of binding density within the striatum, prefrontal cortex and substantia nigra.

Significance: Alterations in the [^{3}H] SR141716A binding in the examined brain areas may represent the specific regions that mediate motor and cognitive dysfunctions in PD via the endocannabinoid system. Our data suggest a critical role of CB1 receptors in treating PD with simvastatin, and implicate CB1 receptors as a potential therapeutic target in the treatment of PD.

Abbreviations: 6-OHDA, 6-hydroxydopamine; 2-AG, 2-arachidonoylglycerol; BDNF, brain derived neurotrophic factor; CB1, cannabinoid receptor type 1; Cg, cingulate cortex; CNS, central nervous system; CO_2, carbon dioxide; Cpudl, caudate putamen dorsolateral; Cpum, caudate putamen medial; Cpuvl, caudate putamen ventrolateral; Cpuvm, caudate putamen ventromedial; DOPAC, 3,4-dihydroxyphenylacetic acid; eNOS, endothelial nitric oxide synthase; FPP, farnesyl pyrophosphate; GABA, gamma-aminobutyric acid; GGP, geranylgeranyl-pyrophosphate; Gpe, globus pallidus externus; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; HVA, homovanillic acid; ICAM-1, intracellular adhesion molecule-1IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; MFB, medical forebrain bundle; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NA, nucleus accumbens; NADPH-oxidase, nicotinamide adenine dinucleotide phosphate oxidase; PD, Parkinson's disease; PFC, prefrontal cortex; ROS, reactive oxygen species; S.E.M, atandard error of mean; SD rats, Sprague Dawley rats; SNc, subsantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; TGF-α, transforming growth factor alpha; TNF-α, tumour necrosis factor alpha.

* Correspondence to: X.-F. Huang, Illawarra Health and Medical Research Institute, School of Medicine, University of Wollongong, Northfield Avenue, NSW 2522, Australia.
** Correspondence to: Q. Wang, Department of Neurology, Director of Neurological Research Lab, The Third Affiliated Hospital of Sun Yat-Sen University, Tianhe Road 600#, Guangzhou, Guangdong 510630, PR China.
E-mail addresses: xhuang@uowmail.edu.au (X.-F. Huang), denniswq@yahoo.com (Q. Wang).
[1] The first three authors contributed equally to this work.

http://dx.doi.org/10.1016/j.lfs.2016.05.005
0024-3205/

1. Introduction

Statins are a hypolipidemic group of drugs that inhibit the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutary-CoA (HMG-CoA) reductase. Due to their cholesterol lowering capability, statins have been a viable option in the treatment of heart diseases, cerebral ischemia, and neurodegenerative diseases [30,63]. As statins suppress HMG-CoA reductase, this actively inhibits other signalling pathways downstream including isoprenoid intermediates, geranylgeranyl pyrophosphate (GGPP) or farnesyl pyrophosphate (FPP) [52]. Suppression of these intermediates would in turn functionally de-activate the GTPases like Rho, Rac and cdc42 which act as molecular switches for various mechanisms. These include lowering cholesterol, suppression of intracellular adhesion molecule-1 (ICAM-1), anti-inflammatory responses, reduction of β-amyloid production, regulation of serum apolipoprotein E levels, anti-thrombotic effects, modification of cognition-related receptors, and augmentation of endothelial nitric oxide synthase (eNOS) [33,37,49,52,57]. More specifically, by attenuating the biological effects of pro-inflammatory mediators such as microglia and cytokines which are known to have a substantial role in neurodegenerative diseases such as Parkinson's disease and stroke, statins have been identified as a potential therapeutic agent [1,5,11,32,57].

Our previous studies have documented statins' effects in a parkinsonian rat model induced by 6-hydroxydopamine (6-OHDA), which is a broadly used PD model. Our results revealed the expression of dopamine D_1 and D_2 receptors in the prefrontal cortex was down-regulated in 6-OHDA-injected rats relative to controls, however simvastatin treatment restored the expression of both dopamine receptors towards control levels [55]. Although, our group among others have examined how statins affect the dopaminergic system in PD, few studies have looked into whether statins treatment could alleviate the dysfunctions of other key receptors that are altered during PD progression. Furthermore, a better understanding of how those receptors malfunction in PD may provide more valuable insight into how the symptoms of PD arise.

Cannabinoid receptor type 1 (CB1) is part of the endocannabinoid system and mainly involved in neurotransmission regulation in many brain regions including the basal ganglia through their abundant expression on GABAergic and glutamatergic synapses [45]. CB1 receptors are primarily located on the pre-synaptic terminals and a part of a retrograde signalling system that becomes activated by the endocannabinoids, anandamide or 2-arachydonoil glycerol (2AG). Though dopaminergic neurons are not in direct contact with CB1 receptors, interactions between the cannabinoid and dopaminergic system have been found through the G protein/adenylyl cyclase signal transduction mechanisms shared by both CB1 and D_1/D_2 dopaminergic receptors [12] and the co-localization of CB1 and D_1/D_2 receptors in striatal neurons [35]. Dopamine can even enhance the release of endocannabinoids from neuronal somas and dendrites, inducing retrograde signalling pathway of CB1 receptors [13,24,34].

With the abundance of CB1 receptors in the basal ganglia and their interactions with multiple neurotransmitters involved in motor or anxiety function, it has been identified that CB1 receptors are significantly altered in various neurological disorders, such as in Parkinson's disease and schizophrenia [4,31]. This study will follow our previous study with chronic simvastatin treatment in 6-OHDA lesioned rats, and investigate how the CB1 receptors are affected following 6-OHDA and simvastatin treatments.

2. Results

This study showed CB1 binding was extensively distributed throughout the brain with a very low background binding. The strongest [^{3}H] SR141716A binding was observed in the cortical areas, striatum, and limbic regions such as the nucleus accumbens (NAc), cingulate cortex (Cg), hippocampus, and substantia nigra (SN). Comparisons were made between the sham and treatment groups.

2.1. Prefrontal cortex (Pfc)

The binding density in the prefrontal cortex revealed significant effects of simvastatin treatment ($F_{2, 69} = 4.493$, $p < 0.05$) and 6-OHDA lesion ($F_{1, 69} = 29.767$, $p < 0.001$), as well as an interaction between these factors ($F_{2, 69} = 19.086$, $p < 0.001$). Compared to the sham group, post hoc comparisons revealed that [^{3}H] SR141716A binding density was significantly down-regulated in the 6-OHDA treated rats (31%, $p < 0.001$ Fig. 2), and in sham rats with high simvastatin treatment (10%, $p < 0.001$). The 6-OHDA-lesioned rats treated with 10 mg/kg/day of simvastatin revealed a significant elevation in the binding levels when compared to rats with 6-OHDA lesion alone (41%, $p < 0.05$, Figs. 2 and 3). Simvastatin treatment with 1 mg/kg/day in 6-OHDA rats did not significantly alter their binding level density when compared to just 6-OHDA lesioned rats.

2.2. Striatum (Cpu)

The binding density in the striatum revealed significant effects of simvastatin treatment only within the Cpudl ($F_{2, 71} = 4.306$, $p < 0.05$) and a trend towards significance in the Cpum ($F_{2, 71} = 3.028$, $p = 0.055$).

In regards to the 6-OHDA lesion, all regions of the striatum revealed a significant difference ($p < 0.05$). The interaction between the two factors revealed a significant effect within the Cpum ($F_{2, 71} = 4.008$, $p < 0.05$), Cpuvl ($F_{2, 71} = 13.241$, $p < 0.001$), ($F_{2, 71} = 7.245$, $p < 0.001$) and a trend towards significant difference in the Cpudl ($F_{2, 71} = 2.973$, $p = 0.058$). Compared to the sham group, post hoc comparisons revealed that the [^{3}H] SR141716A binding levels were significantly down-regulated in the rats with only 6-OHDA lesion in all regions of the striatum ($p < 0.05$, Figs. 2 and 3). The 6-OHDA-lesioned rats treated with 10 mg/kg/day of simvastatin showed a significant elevation in all the stratum binding densities when compared to rats with 6-OHDA lesion alone ($p < 0.05$). No significant difference was observed with 6-OHDA treated rats with 1 mg/kg/day of simvastatin when compared to 6-OHDA lesioned rats alone.

2.3. Nucleus accumbens (NAc)

The binding density of the nucleus accumbens did not show any significant changes after the treatment ($F_{2, 71} = 2.405$, $p > 0.05$). However, there were significant changes with lesioning ($F_{1, 71} = 6.356$, $p < 0.05$) and interactions between these factors ($F_{2, 71} = 8.802$, $p < 0.001$). Compared to the sham group, post hoc comparisons analysis revealed that [^{3}H] SR141716A binding levels were significantly lower in rats with only 6-OHDA lesion (14%, $p < 0.05$, Figs. 2 and 3). The 6-OHDA-lesioned rats treated with 10mg/kg/day of simvastatin revealed a significant elevation in this binding level when compared to rats with 6-OHDA lesion alone (23%, $p < 0.001$, Figs. 2 and 3). No significant difference was observed with 6-OHDA treated rats with 1 mg/kg/day of simvastatin when compared to 6-OHDA lesioned rats alone.

2.4. Cingulate cortex (Cg)

Significant effects of treatment on binding levels in the cingulate cortex were revealed ($F_{1, 71} = 4.364$, $p < 0.05$) and lesion ($F_{2, 71} = 16.608$, $p < 0.001$), as well as significant interactions between these factors ($F_{2, 71} = 6.997$, $p < 0.005$). Relative to the sham group, post hoc comparisons revealed that the [^{3}H] SR141716A binding levels were significantly down-regulated in the 6-OHDA lesioned rats (25%, $p < 0.001$; Figs. 2 and 3). The 6-OHDA-lesioned rats treated with 10 mg/kg/day of simvastatin revealed a significant elevation in the binding levels when compared to rats with 6-OHDA lesion alone (26%, $p < 0.001$, Figs. 2 and 3).