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SIMVASTATIN REVERSES THE DOWNREGULATION OF M1/4 RECEPTOR BINDING IN 6-HYDROXYDOPAMINE-INDUCED PARKINSONIAN RATS: THE ASSOCIATION WITH IMPROVEMENTS IN LONG-TERM MEMORY

Q. WANG, [a,b*†] **X. WEI,** [a†] **H. GAO,** [a†] **J. LI,** [a] **J. LIAO,** [a] **X. LIU,** [a] **B. QIN,** [a] **Y. YU,** [b] **C. DENG,** [b] **B. TANG** [c] **AND X.-F. HUANG** [b]

[a] *Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, Tianhe Road 600, Guangzhou, Guangdong 510630, PR China*

[b] *Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, NSW 2522, Australia*

[c] *Department of Neurology, Xiangya Hospital of Central South University, Changsha, Hunan 410008, PR China*

Abstract—*Background:* It is believed that muscarinic M1/4 receptors are closely correlated to the dopaminergic system and are strongly involved in the pathogenesis of Parkinson's disease (PD). In addition to regulating lipid metabolism and protection from stroke, statins have been used to regulate the declined cognition. We aimed to explore the regional changes in M1/4 receptors in the 6-hydroxydopamine (6-OHDA)-lesioned rat brain.

***Methods:* PD rat model was set up by injecting 6-OHDA into the unilateral medial forebrain bundle; while simvastatin (10 mg/kg/day) or saline was orally administrated for 3 weeks, respectively. Long-term memory was measured using the Morris water maze. [^{3}H]pirenzepine binding autoradiography was applied to investigate the alterations of M1/4 receptors in the PD rat brains.**

***Results:* 6-OHDA induced long-term memory deficits along with downregulation of M1/4 receptors in the hippocampus, the medial amygdala, the posteromedial cortical and the piriform cortex; simvastatin administration significantly ameliorated the impaired memory and reversed the downregulation of M1/4 receptors in the examined brain regions. Profound positive correlations were verified between the decline in long-term memory activity and the restoration of M1/4 receptors in different brain regions after simvastatin treatment.**

***Conclusions/significance:* Our results provide strong evidence that M1/4 receptor modulation after simvastatin administration did, at least partially, contribute to the improvement in the long-term memory in 6-OHDA-induced PD rats. These results provide a possible mechanism for simvastatin treatment in psycho-neurological diseases such as PD via M1/4 receptors.**

Key words: Parkinson's disease, simvastatin; M1/4 receptors; memory.

INTRODUCTION

The cholinergic system and its neurodegeneration associated with Neurodegenerative diseases such as Parkinson's diseases (PD) and Alzheimer's disease (AD) have obtained considerable attention. Muscarinic receptors, one of the important elements in the cholinergic system, have been widely explored. Muscarinic receptors are G-protein-coupled receptors (GPCR) that include five subtypes M1–M5 (Bymaster et al., 2003). Among these five subtypes, muscarinic M1 receptors that are localized to the postsynaptic membrane are the most studied and abundant in the cerebral cortex, the amygdala, the caudate putamen (CPu), and the hippocampus (Wang et al., 2008). Increasing evidence indicates important roles for M1 receptors in learning and functional memory regulation among the various neuropsychological disorders (Auld et al., 2002; Hu et al., 2010; Digby et al., 2012). In aged rats with a decline in memory and adult-onset cognitive dysfunction, the densities of M1 and M4 receptors were profoundly decreased in brain regions including the Cornu Ammonis area 1 (CA1) and the frontal and occipital cortices (Tayebati et al., 2006). In Alzheimer's and PD patients with impaired cognitive function, the expression of muscarinic receptors was found to be

*Correspondence to: Q. Wang, Department of Neurology, Director of Neurological Research Lab, The Third Affiliated Hospital of Sun Yat-Sen University, 600 Tianhe Road, Guangzhou, Guangdong 510630, PR China. Tel: +86-20-85253295; fax: +86-20-85253117.
E-mail addresses: denniswq@yahoo.com, wqdennis@gmail.com (Q. Wang).

† The same contribution.

Abbreviations: ABC, avidin–biotin–peroxidase complex staining system; Acb, accumbens nucleus; AD, Alzheimer's disease; AI, agranular insular cortex; Amy, amygdala; ANOVA, analysis of variance; Arc, arcuate hypothalamic nucleus; Cg, cingulate cortex; CPu, caudate putamen; DAB, diaminobenzidine; DMH, dorsomedial hypothalamic nucleus; DP, Di-*N*-butyle phthalate in xylene; Ent, entorhinal cortex; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; Hip, hippocampus; LDL, low-density lipoprotein; M1, primary motor cortex; M1/4 receptor, muscarinic1/4 receptor; Me, medial amygdala; MFB, medial forebrain bundle; MWM, morris water maze; Pir, piriform cortex; PD, Parkinson's disease; PMCo, posteromedial cortical amygdala; PRh, perirhinal cortex; RSG, retrosplenial granular cortex; SNpc, compact part of substantia nigra; SNr, reticular part of substantia nigra; TH, tyrosine hydroxylase; Tha, thalamus; Tu, olfactory tubercle; VDB, vertical limb of the diagonal band; VMH, ventromedial hypothalamus; VTA, ventral tegmental area; 6-OHDA, 6-hydroxydopamine.

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decreased in the brain (Rodriguez-Puertas et al., 1997; Araki et al., 2000).

Statins, hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, were normally recognized to lower plasma cholesterol. In addition to lowering cholesterol, statins have gradually been used to reduce the risk of heart attack, cerebral ischemia, and have been demonstrated to exert potential beneficial effects in different neurological diseases (Wang et al., 2007; Keener and Sanossian, 2008; van der Most et al., 2009; Sugawara et al., 2011; Ayer et al., 2013). Although the precise mechanisms are unclear and contrasting results have been observed (Reiss and Wirkowski, 2007; Becker et al., 2008), additional studies have found that statins display important effects on cognitive-related neurological diseases such as PD (Wu et al., 2008; van der Most et al., 2009; Wang et al., 2011; Lee et al., 2013; Xu et al., 2013). If statins would have a potentially useful impact on impaired memory regulation in the progression of PD, this presents an interesting challenge.

Besides being characterized by typical disturbances of the dopaminergic system, PD patients also display widely unbalances in other systems including cholinergic system (Gubellini et al., 2004; Lester et al., 2010; Xu et al., 2012). It has been indicated that the interruption of the dopaminergic system may influence the cholinergic system in PD (Andersson et al., 2010; Lester et al., 2010). Our studies and Selley (Selley, 2005; Wang et al., 2005) have shown that simvastatin regulated dopaminergic systems including receptors and transmission in the brain. Also, our study found the alterations of *N*-methyl-D-aspartic acid (NMDA) and M1/4 receptor in the brains following simvastatin treatment (Wang et al., 2008, 2009b; Yan et al., 2011). Whether the application of simvastatin in parkinsonian rats also influences the expression of M1 receptors raises an interesting question.

Based on the information above, we propose that simvastatin may reverse the decline in M1/4 receptors in memory-related regions of the brain in 6-hydroxydopamine (6-OHDA)-induced PD rats. To test this hypothesis, [^{3}H]pirenzepine binding autoradiography was applied to verify the reactions of muscarinic receptors M1/4 to 3-week treatment of simvastatin in the 6-OHDA-induced PD rat model. Besides, we also investigated the impairment of memory in this PD rat model, as well as the influence of simvastatin on declined memory in the PD rat model. The current study provides a possible association of simvastatin treatment with M1/4 receptors in the parkinsonian rat brain.

EXPERIMENTAL PROCEDURES

Animals and drug treatments

Twenty-two Sprague–Dawley rats (male, 230–250 g/rat) were purchased from the Animal Resources Centre (Perth, Western Australia, Australia) and kept in specifically environmental conditions as previously described (Li et al., 2010). Rats were housed for 1 week to be accustomed to the new environment before experiments start. The rats were randomly assigned to groups with a total of sixteen rats for the 6-OHDA-lesioned group, among which eight of the rats were administered with simvastatin (10 mg/kg/day) orally and the rest of the rats were orally administrated with saline. One 6-OHDA-lesioned rat receiving saline died after surgery. In the control group, six rats received 0.9% saline for 3-week oral administration as the vehicle control. After 3 weeks of 6-OHDA lesion, all rats were sacrificed to examine M1/4 receptor binding. This study was approved by the local Animal Ethics Committee, and followed the instructions in compliance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals* (*NIH Publications* No. 80-23) revised 1996 guidelines and National Health and Medical Research Council (NHMRC) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004).

6-OHDA-lesioned parkinsonian rats

The 6-OHDA-lesioned rat was created as described in Yan's work (Yan et al., 2011). Briefly, the rats were anesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine (Troy laboratories Pty, Ltd., Smithfield, Australia). Four microliters 6-OHDA was diluted in the normal saline (8 μg/μl in normal saline containing 0.2 mg/ml ascorbic acid, Sigma–Aldrich, St. Louis, MO, USA) and were unilaterally injected into the medial forebrain bundle (MFB) (0.8 μl/min; at anteroposterior (AP) −4.4 mm, mediolateral (ML) −1.4 mm and dorsoventral (DV) −7.8 mm, from Bregma (Paxinos and Watson, 1997). The control group was orally administrated with the 0.9% saline vehicle, and received the 0.9% saline injection into the MFB. These animal procedures were conducted according to the protocols set up by the University of Wollongong, adapted from Howard-Jones (1985).

Morris water maze (MWM)

Three weeks after the 6-OHDA lesion was performed, rats were tested in the MWM to assess the level of long-term memory. The MWM was described in previous studies (Andringa et al., 2000; Wisman et al., 2008). Briefly, the pool dimensions were a diameter of 185 cm and a water depth of 30 cm, and the water temperature was 21 °C. A hard plastic circular cover that fits inside the wall of pool was used as a protective sheath so that the rats would not scratch the rubber pool material. Four starting positions were conventionally indicating east, south, west, and north, which divided the pool into four quadrants. A target platform (10 × 10 cm) was placed midway between the center and the western point. The platform consisted of transparent plexiglass and was submerged 1.5 cm beneath the surface of water. Rats were tested 2 days in succession, with two training trials conducted in the first day for the learning phase. On the second day, the test trial was performed to test the long-term memory. The distance that the rat traveled and the time that the rat took to reach the platform was recorded by a camera placed 2 m above the water maze. Distance