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Effects of high dose of simvastatin on levels of dopamine and its reuptake in prefrontal cortex and striatum among SD rats

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Abstract

Statins are increasingly being used for the treatment of a variety of conditions beyond their original indication for cholesterol lowering. We previously reported that simvastatin increased dopamine receptors in the rat prefrontal cortex [Q. Wang, W.L. Ting, H. Yang, P.T. Wong, High doses of simvastatin upregulate dopamine D_1 and D_2 receptor expression in the rat prefrontal cortex: possible involvement of endothelial nitric oxide synthase, Br. J. Pharmacol. 144 (2005) 933–939] and restored its downregulation in a model of Parkinson's disease (PD) [Q. Wang, P.H. Wang, C. McLachlan, P.T. Wong, Simvastatin reverses the downregulation of dopamine D1 and D2 receptor expression in the prefrontal cortex of 6-hydroxydopamine-induced Parkinsonian rats, Brain Res. 1045 (2005) 229–233]. Here we explore the effects of simvastatin treatment on tissue dopamine content and reuptake. Sprague–Dawley rats were given simvastatin (1 and 10 mg kg^{-1} day^{-1}, p.o.) for 4 weeks. Brain tissue from prefrontal cortex and striatum were taken out for dopamine content and its reuptake. Using high-performance liquid chromatographic-mass spectrometer (HPLC-MS), simvastatin (10 mg kg^{-1} day^{-1}) was found to increase dopamine content by 110% in the striatum but decreased by 76% in the prefrontal cortex compared with the saline treated group. Dopamine (DA) reuptake was unchanged in both brain regions. These results suggest that chronic treatment with high dose of simvastatin may affect DA tissue level in prefrontal cortex and striatum without changing on DA reuptake. This may have important clinical implications in psychiatric and striatal dopaminergic disorders.

Keywords: Hydroxymethylglutaryl-coenzyme reductase inhibitors; Simvastatin; Reuptake; DA; Rat striatum; Prefrontal cortex

Statins reduce serum low-density lipoprotein (LDL) cholesterol by inhibiting the rate-limiting enzyme, hydroxymethylglutaryl-coenzyme reductase, in cholesterol synthesis. These drugs are now widely used clinically for the prevention of atherosclerotic disease. Meta-analyses of data from major clinical trials have shown that the risk of ischaemic heart disease events is reduced by 60% and stroke by 17% [17]. Statins are also being recognized to have potential medical applications such as in peripheral arterial disease, end-stage renal disease, diabetes mellitus, and Alzheimer's disease (AD) [22,33]. In animal studies, statins also appear to be beneficial in traumatic brain injury [20] and neuroinflammation [2]. Despite growing evidence for a role of statins in central nerve system (CNS) diseases such as stroke and AD, there is relatively little knowledge of their direct neurological effects on central dopaminergic disorders.

Dopamine (DA) is a predominant catecholamine neurotransmitter, and acts as a key neurotransmitter in the brain. It is derived from the amino acid L-tyrosine, which is hydroxylated by tyrosine hydroxylase to L-dopa and subsequently decarboxylated by L-aromatic amino acid decarboxylase to form DA. It is believed that DA release sites exist outside of the synaptic cleft [7]. Once release from neuronal membranes, DA diffuses in the extracelluar fluid from which it is slowly cleared as a result of reuptake and metabolism.

As a neurotransmitter, DA activity can regulate a variety of behaviors and brain functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation [3]. Therefore, increases or decreases in DA release directly leads to changes in dopaminergic functions in the brain with important clinical implications. Reduction of

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DA in the brain is believed to cause changes in motor function and cognition. The loss of DA in the basal ganglia is associated Parkinson's disease (PD) [8,9] with the greatest reduction of tissue content of DA in the putamen [15]. The DA content in the caudate nucleus was found to decrease not only in PD but also in aged human brain [4], which correlates with age-related reduction in motor activity.

Evidence indicates that DA dysfunction in animal brain could also lead to cognitive and psychological disorders [12]. Studies by Suzuki et al. [30] showed that methamphetamine sensitized rats produced an obvious increase in DA release in the amygdala, while studies by Konstandi et al. [16] in mice showed that stress produced a decrease in DA level in the hypothalamus and an increase in DA level in the amygdala. In addition, this study in rats also showed that stress caused a decrease in DA level in the striatum but an increase in the locus coeruleus [16].

In our previous study, we found that chronic treatment with simvastatin at dosage of $10\,mg\,kg^{-1}\,day^{-1}$ but not $1\,mg\,kg^{-1}\,day^{-1}$ increased dopamine receptors D_1 and D_2 in the prefrontal cortex but not in the striatum among naïve and 6-OHDA lesioned rats [34,35]. We, therefore, decided to investigate if simvastatin may affect DA content and presynaptic DA reuptake in different parts of the brain.

DA and internal standards were purchased from Sigma–Aldrich. High-performance liquid chromatographic (HPLC) grade methanol and acetonitrle were purchased from Merck Darmstadt, Germany. HPLC system consisted of an Agilent 1100 Binary pump quipped with an Agilent 1100 autosampler injector with 100 loop and 1100 column oven set at 23 °C (Agilent Technologies, Germany). HPLC-mass spectrometer (MS) analysis was performed using an API 2000 triple-quandrupole mass spectrometer (Applied Biosystems, MDS SCIEX, Ontario, Canada). Phosphate-buffered Krebs medium [23] was aerated with 95% O_2 and 5% CO_2 1 h before doing the DA reuptake measurement. Dopamine hydrochloride and all of the chemical reagents were purchased from Sigma–Aldrich.

Male Sprague–Dawley rats (280–300 g) were obtained from the University Laboratory Animal Centre and housed four or five per cage with food and water available ad libitum under natural light–dark cycle (approximately 12–12 h). All experiments were carried out in accordance with the guidelines set by the National University of Singapore [10]. Simvastatin (1 and $10\,mg\,kg^{-1}\,day^{-1}$) (Ranbaxy Laboratories, Dewas, India) suspended in 1.5 ml saline was administered by gavage feed for a period of 4 weeks. Brain tissue from prefrontal cortex and striatum was taken out after 4-week oral administration for HPLC-MS and reuptake measurements. Control rats received only saline.

At the end of the 4-week treatment period, blood samples were collected for the determination of fasting (10–12 h) serum cholesterol and triglyceride levels. Triglyceride and cholesterol assay kits were obtained from ThermoTrace (Noble Park, Australia). Sampling of brain tissue and HPLC-MS measurement were modified according to Zhang et al. [37]. Briefly, rats were sacrificed with CO_2 and brains were removed. The prefrontal cortices and striata were isolated and immersed immediately in liquid nitrogen and stored at −80 °C until used for HPLC analysis. The prefrontal cortex samples were homogenized after adding 0.1N $HClO_4$ solutions in the ratio of 1 ml per 0.1 g brain tissues into the plastic tubes. Striata tissues were processed the same way but with a ratio of 1 ml of 0.1N $HClO_4$ per 0.05 g tissue. The tissues were homogenized for 30 s and the resulting homogenates were centrifuged for 5 min at $15000 \times g$. Five hundred microliters supernatant was collected for quantification of DA. The supernatants were spiked with 10 μl of 1,1,2,2-D4 dopamine hydrochloride solution used as an internal standard and alkalized using 600 μl of glycine buffer solution. The solid-phase extraction (SPE) was performed using Bond Elut-PBA column (1 ml). The columns were conditioned subsequently with 1 ml of methanol, and 1 ml of 10 mM ammonium acetate, adjusted to pH 8.0 with ammonium solution. The alkalized supernatants were loaded and then washed with 1 ml of 10 mM ammonium acetate and followed by 1 ml of Milli-Q water. The columns were dried under vacuum for 5 s. The columns were eluted with 200 μl of trifluoroacetic acid (0.1) for three times. The first 200 μl of elute was disposed and the following second and third 200 μl of elute were collected and combined in 1.7 ml centrifuge tubes. The tubes were centrifuged at $5000 \times g$ for 5 min. Hundred microliters of elute supernatant was transferred injection vial (250 μl) for analysis. Ten microliters of elute supernatant was injected into HPLC-MS system and immediately analyzed for DA.

DA reuptake was assayed as previously described [23] with some modifications. Synaptosomal membranes were incubated with Kreb-Ringer buffer (1 ml, composition in mM: NaCL 118, Na_2HPO_4 16.2, KCL 4.7, $CaCL_2$ 1.8, $MgSO_4$ 1.2, glucose 5.8 was aerated with 95% O_2 and 5% CO_2 1 h before doing the DA reuptake measurement) at 37 °C for 5 min pre-incubation before the addition of [^{3}H] DA (0.5 μCi, 0.1 ml, final concentration of 1 μM). Ten minutes later, reuptake was stopped by rapid filtration under vacuum through a Whatman GF/C cellulose nitrate filters (0.25 μm pore size) which were then washed twice with 3 ml ice-old saline. The filters were placed in liquid scintillation vials containing 7.5 ml of Picofluor (Amersham), and then radioactivity retained on the filters was determined by liquid scintillation spectrometry (Beckman LS 3801; Beckman Instruments, Fullerton, CA). Nonspecific reuptake was determined in the presence of 10 μM nomifensine in the essay buffer [28]. Specific reuptake was defined as total uptake minus the nonspecific uptake.

All statistical procedures were performed using a paired *t*-test and one-way analysis of variance (ANOVA) followed by two way *t*-test (SPSS for Windows v12) to determine significant difference with the significant threshold set at $P = 0.05$ level. All data were shown mean ± S.E.M.

Fig. 1 shows that simvastatin treatment at 1 and $10\,mg\,kg^{-1}\,day^{-1}$ did not significantly alter serum cholesterol and triglyceride levels in rats.

Since dopamine receptor D_1/D_2 expression was measured in both prefrontal cortex and striatum in previous work, tissue level of DA was also measured in these two regions. It was found that after simvastatin treatment ($10\,mg\,kg^{-1}\,day^{-1}$) DA tissue level decreased by 76% in prefrontal cortex but increased by 110% in striatum compared to the saline treated control