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SIMVASTATIN INCREASES EXCITABILITY IN THE HIPPOCAMPUS VIA A PI3 KINASE-DEPENDENT MECHANISM

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Abstract—Simvastatin is an HMG-CoA reductase inhibitor commonly used in the clinic to treat hypercholesterolemia. In addition, simvastatin has been shown to cross the blood–brain barrier and pleiotropic effects of simvastatin have been reported including anti-inflammatory properties, enhancement of neurite outgrowth, and memory enhancement properties. However, little has been reported on the effects of simvastatin on basal synaptic transmission and neuronal excitability. Here we report that simvastatin increases the fEPSP, the N-methyl-D-aspartate (NMDA) receptor-mediated fEPSP using extracellular recordings in the dendritic region of the CA1 of hippocampal slices taken from 8-week-old C57Black6J mice. In addition, we found that simvastatin perfusion causes a change in the input/output curve and a decrease of the paired-pulse facilitation ratio, indicating respectively an increase of the neuronal excitability and neurotransmitter release. We have also observed that acute application of simvastatin increased the amplitude of the compound action potential in the CA1 region. Notably, using LY294002, we have demonstrated that this effect was PI3K dependent and was occluded if the animals had previously received a diet supplemented with simvastatin. We have finally shown that the simvastatin-mediated increase of the compound action potential amplitude was also present in hippocampal slices from aged mice.

Key words: mouse, cholesterol, simvastatin, hippocampal slice, *in vitro*.

INTRODUCTION

Statins have been prescribed to treat hypercholesterolemia and to reduce plasma levels of low-density lipoproteins (LDLs) to prevent cardiovascular disease. The main mechanism of action is via inhibition of 3-hydro xy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, a rate-limiting enzyme of cholesterol synthesis (Endo, 1992; Tobert, 2003). Each HMG-CoA reductase inhibitor has a different blood–brain barrier permeability due to specific chemical properties. Lipophilic simvastatin, lovastatin and hydrophilic pravastatin, all contain a carboxylic acid group which appears to facilitate access to the blood–brain barrier (BBB); simvastatin being one of the more permeable agents. The proton/monocarboxylate transporter is thought to be the mode of statin delivery (Saheki et al., 1994). There are many reported pleiotropic effects of statins. For example, chronic statin treatment can enhance memory (e.g., atorvastatin or simvastatin, (Grupe et al., 2006; Li et al., 2006; Lu et al., 2007), decrease inflammatory cytokine production (Balduini et al., 2003), improve cerebral blood flow to a site of injury (Chen et al., 2003) and reduce deficits in long-term potentiation in a mouse model of Alzheimer's disease (AD) (Metais et al., 2014). Moreover statins can enhance neurogenesis in the dentate gyrus (Chen et al., 2003; Lu et al., 2007) in addition to promoting angiogenesis and neurite outgrowth (Pooler et al., 2006). Anxiolytic-like effects of simvastatin have also been reported (Wang et al., 2009). In 2008, Kannan et al. demonstrated that chronic mevastatin treatment on mouse primary cortical neurons increased the voltage-gated sodium current and decreased N-methyl-D-aspartate (NMDA) currents. Acute incubation of mouse hippocampal slices in simvastatin (10 μM) has been shown to increase the levels of phosphorylated Akt, which was linked with enhanced long-term potentiation (LTP) (Mans et al., 2010). While the effects of acute statin treatment on hippocampal LTP have been reported, there has been no report of any effect on baseline synaptic transmission or axonal excitability.

Here, we have used extracellular recordings in the CA1 region of c57 black6 mouse hippocampal slices to examine the effects of acute simvastatin treatment on synaptic transmission and on neuronal excitability. We report that bath application of simvastatin to naïve slices can rapidly and significantly increase synaptic transmission directly via a PI3-kinase-dependent mechanism. This effect may be partially responsible for the previously reported simvastatin-mediated increased levels of LTP (Mans et al., 2010). We found that simvastatin also increased the amplitude of the compound action potential (cAP) recorded in the *alveus* in a PI3K-dependent manner. This effect was occluded in slices from animals that previously received a simvastatin supplemented diet. In addition, we have previously reported differential effects of chronic simvastatin treatment in the APPswe/PS1dE9 mouse model of AD. Here we also demonstrate that naïve slices from this

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Abbreviations: AD, Alzheimer's disease; BDNF, brain derived neurotrophic factor; cAP, compound action potential; DNQX, 6,7-dinitroquinoxaline-2,3-dione; fEPSPs, field excitatory post synaptic potentials; HFS, high-frequency stimulation; HMG CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate; PIP3, phosphatidyl inositol 3 phosphate; PPF, paired-pulse facilitation.

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mouse AD model fail to show an increase in synaptic transmission following acute simvastatin treatment.

EXPERIMENTAL PROCEDURES

Animals and diet

In our experiments, we used c57 black6 mice that were either 7–9 weeks of age, purchased from Harlan UK or 18-month-old bred in the Conway Institute Animal Facility, University College Dublin. Male and female mice were used in this study. Mice were housed in the animal facility with a dark/light cycle of 12 h, fed with chow and water *ad libitum*. To study the chronic effects of simvastatin, c57 black6 mice were fed with chow pellets supplemented with 0.04% simvastatin, representing a daily dose of 40 mg kg^{-1}. At 12 months, animals were treated for 6 months (13–18 months inclusive). All experiments were carried out in accordance with guide lines and under licence from the Department of Health, Ireland (86/609/EEC).

Drugs

Simvastatin was purchased from Molekula, 6,7-dinitroquinoxaline-2,3-dione (DNQX), LY294002 and AP5 were obtained from Tocris. To activate simvastatin, it was prepared by dissolving in 45% NaOH and 55% ethanol mix. Just prior to bath application the pH was balanced with HCL.

Electrophysiology and hippocampal slices

Hippocampal slices, 400-μm thick were prepared as previously described (Metais et al., 2014). Electrodes were pulled from borosilicate capillary glass (GC150 F-10, Havard apparatus), using a horizontal puller (DMZ universal puller, Germany). Electrodes (2-5 MΩ) were filled with aCSF. (NaCl 119 mM; D-glucose 11 mM; $NaHCO_3$ 26 mM; KCl 2.5 mM; $MgSO_4$ 1 mM; $CaCl_2$ 2.5 mM; NaH_2PO_4 1 mM). The voltage signal was filtered at 5 kHz and stored for off-line analysis using a personal computer interfaced with a CED/National Instruments A/D board and WinCP software (J. Dempster, Strathclyde University). The Shaffer-collateral pathway was stimulated using a monopolar electrode (FHC, Bowdoin, USA) at 0.033 Hz (duration: 100 μs), the return electrode was a silver/silver chloride wire placed in the recording bath. Extracellular field excitatory post synaptic potentials (fEPSPs) were recorded in the *stratum radiatum* of the CA1 at 30 °C. Paired-pulse facilitation was examined using paired stimuli delivered with an inter-stimulus interval of 50 ms. To examine the cAP, the CA1 axons within the *alveus* were activated antidromically and the cAP was recorded in the CA1 cell body region. Signals were amplified by a HS2A headstage (Molecular Devices, USA) connected to an Axoclamp 2B system (Molecular Devices, USA) and a Brownlee 410 Precision preamplifier. A Master 8 (AMPI) timer was used to deliver and time the stimulus trigger. Stable field EPSPs or cAPs were recorded for 20 min, at 40–50% maximum response prior to bath application of simvastatin or PI3-kinase antagonists. When LTP was induced, control EPSPs were recorded for 20 min prior to the application of high-frequency stimulation (HFS). LTP was induced using two trains of stimuli at 100 Hz for 1 s, with an inter-train interval of 30 s. Following the application of HFS, the synaptic response was recorded for a further period of 60 min. Statistical analysis was performed using either paired/unpaired *t*-tests or ANOVA. All results are presented as mean ± SEM. The "*n*" numbers quoted refer to the number of slices used. Control and test experiments in a given section were conducted on the same day on slices from the same animal.

RESULTS

Acute application of simvastatin caused a significant increase in the fEPSP slope

The effects of acute application of simvastatin were investigated by applying the agent directly to the perfusion reservoir. Following a period of stable baseline recording, simvastatin (10 μM) caused a gradual increase in the fEPSP slope. Following an application time of 35 min there was a significant increase compared to baseline values (99.33 ± 0.1% to 118.3 ± 4.1%, (n = 6), $p < 0.05$; Fig. 1A). The effects of a higher concentration of simvastatin (35 μM) were also investigated and found to cause a rapid and significant increase in the fEPSP slope. When simvastatin had been perfused for 35 min, the synaptic response increased significantly from baseline values (100.2 ± 0.3% to 160.1 ± 13.5%, (n = 11), $p < 0.05$; Fig. 1A). There was however no observed increase in the synaptic response on perfusion of the vehicle alone (98.1 ± 0.72% to 99.43 ± 1.24% (n = 4), $p > 0.05$; Fig. 1). The effect of simvastatin appeared to be concentration-dependent as the higher concentration (35 μM) increased the fEPSP to a greater extent than 10 μM simvastatin ($p < 0.05$, Fig. 1B). The simvastatin-mediated increase in synaptic transmission could also be observed in the presence of 50 μM AP5 (data not shown).

Simvastatin alters the input/output curve and Paired-Pulse Facilitation Ratio in the CA1 region

Application of a range of increasing stimulus intensities produced an incremental increase in the fEPSP slope in all slices. Following acute application of simvastatin (35 μM) there was a leftward shift of the input/output curve. The stimulus intensity that induced 50% of the maximal response was lower following the application of simvastatin (see Fig. 2A). To examine the potential effects of simvastatin on transmitter release we examined paired-pulse facilitation (PPF). Following a stable baseline recording period, acute perfusion of simvastatin (35 μM) significantly decreased the PPF ratio compared to control conditions (1.47 ± 0.06 to 1.30 ± 0.06; (n = 12), $p < 0.05$; Fig. 2B) measured 35 min after the application of simvastatin to the perfusate.

NMDA receptor-mediated field EPSPs were also increased by simvastatin

The effects of simvastatin on NMDA-mediated fEPSPs were also investigated. Following a 20-min period of