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Effects of HMG-CoA reductase inhibitors on learning and memory in the guinea pig

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ABSTRACT

Statins reduce the risk of death from cardiovascular disease in millions of people worldwide. Recent pharmacovigilance data has suggested that people taking statins have an increased risk of psychiatric adverse events such as amnesia and anxiety. This study aimed to investigate the possibility of statin-induced amnesia through animal models of memory and learning. We conducted extracellular field recordings of synaptic transmission in area CA1 of hippocampal slices to examine the effects of acute cholesterol lowering with lipid lowering drugs. We also assessed the effect of six weeks of simvastatin (2 mg/kg/d) and atorvastatin (1 mg/kg/d) treatment using the Morris water maze. Long Term Potentiation (LTP) was significantly diminished in the presence of 3 μM atorvastatin or simvastatin and by the cholesterol sequestering agent methyl-β-cyclodextrin (MBCD). The effects were reversed in the MBCD but not the statin treated slices by the addition of cholesterol. In the water maze, statin treatment did not cause any deficits in the first five days of reference memory testing, but statin treated guinea pigs preformed significantly worse than control animals in a working memory test. The deficits observed in our experiments in water maze performance and hippocampal LTP are suggestive of statin induced changes in hippocampal plasticity. The effects on LTP are independent of cholesterol regulation, and occur at concentrations that may be relevant to clinical use. Our results may help to explain some of the behavioural changes reported in some people after beginning statin treatment.

1. Introduction

Despite their widespread use, it is not known definitively whether 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, referred to as statins influence brain function and behaviour. Data from adverse reaction monitoring programs have indicated that statins may cause neurological symptoms for some people (Tatley and Savage, 2007) and so there would seem to be a strong need for experimental evidence to answer the question either way. Statins have been in use for 26 years, and evidence is now overwhelming that their use has had a major and positive impact on cardiovascular disease (Lytsy et al., 2012; Taylor et al., 2013). Despite the existence of ample experimental data pertaining to common adverse drug reactions (ADRs) such as myalgia and despite recurrent concerns about the effects of statins on mental health (Tatley and Savage, 2007; Tuccori et al., 2008), the potential of statins to cause adverse effects on the brain remains poorly studied. Therefore, in this study we set out to test the effects of statins on brain function both *in vivo* and *ex vivo*, using a species (guinea pig) that has a similar lipid metabolism profile to humans (Fernandez and Volek, 2006; Madsen et al., 2008).

Observational evidence from the clinical use of statins led us to carry out these investigations (Orsi et al., 2001; Padala et al., 2012; Parker et al., 2010; Wagstaff et al., 2003). Pharmacovigilance authorities that have identified signals to suggest that statins may induce psychiatric adverse drug reactions in some patients and have indicated that statins may induce amnesia (King et al., 2003; Tatley and Savage, 2007). The New Zealand Pharmacovigilance Centre (NZPhvC) reported an increase in the number of statin-induced psychiatric adverse events over 2000–2005 (Tatley and Savage, 2007). Up to 2005, 21% of all reported simvastatin-induced ADRs were psychiatric. Importantly, of the 203 psychiatric reports received, 40 reports describe how the psychiatric condition regressed upon withdrawal of the statin and recurred upon re-challenge. Some of the psychiatric conditions reported were mood disorders (including aggression), cognitive disorders (amnesia), sleep disorders (insomnia, paroniria-disturbed sleep often with disturbing nightmares) and perception disorders (hallucinations).

Testing statins in animal models can be challenging as lipid metabolism differs between species. For example, Baytan et al. (2008) administered 4 weeks of 10 mg/kg/day simvastatin treatment to Sprague Dawley rats, which resulted in impaired

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performance in the Barnes maze test of spatial memory (Baytan et al., 2008). But lipid metabolism in rats differs from humans, and statin metabolism is extremely efficient in rats, such that clinically unrealistic doses are required in order to alter blood lipid profiles (Maggo et al., 2012). Other studies have employed guinea pigs; for example, Madsen et al. (2008) found total cholesterol and LDL-C reductions with increasing doses of statins. We previously (Maggo et al., 2012) found that oral delivery of clinically relevant doses of statins could alter guinea pig blood lipid profiles in a way that closely resembles the changes seen in humans. For this reason, guinea pigs were used throughout our experiments.

2. Materials and methods

All methods were approved by the University of Otago Animal Welfare Committee. Male, albino Dunkin–Hartley guinea pigs (250–300 g at start of the investigation) were sourced from the University of Otago's animal breeding facility and housed in cages of three with free access to food and water. Food consisted of standard dry guinea pig food pellets supplemented with cabbage leaf. Room temperature was maintained at 21–24 °C with a 12 h light/dark cycle. Animals were handled daily and allowed to acclimatise for one week before experimentation.

We used hippocampal long term potentiation (LTP) to study synaptic plasticity, and investigated whether acute bath application of statins (i.e., *in vitro)* affect hippocampal LTP in area CA1 of the hippocampus. We also investigated the effects of six weeks of oral statin administration on spatial memory task using the Morris water maze (MWM), and the effects of this statin treatment LTP expression in dissected hippocampi (i.e., *ex vivo)*. Furthermore, *ex vivo* hippocampal tissue was collected and prepared for western blotting analysis of glutamatergic receptor subunits involved in LTP.

2.1. Electrophysiology

2.1.1. Dissection and maintenance of hippocampal slices

Methods were adapted from those described in Sari and Kerr (2001) and Mockett et al. (2007). Male albino guinea pigs (Dunkin–Hartley) aged 6–8 weeks were briefly exposed to carbon dioxide and rapidly decapitated. Brains were then rapidly dissected out and immersed into ice cold artificial cerebrospinal fluid (aCSF) (124 mM NaCl, 3.2 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose) saturated with carbogen (95% O_2/5% CO_2). Hippocampi from each hemisphere were dissected out and immersed into ice-cold aCSF. Hippocampal slices (400 μm transverse sections) were obtained using a Stoelting tissue slicer (Stoelting, Wood Dale, Illinois, USA) and maintained in an *in vitro* brain slice holding chamber at 31 °C,

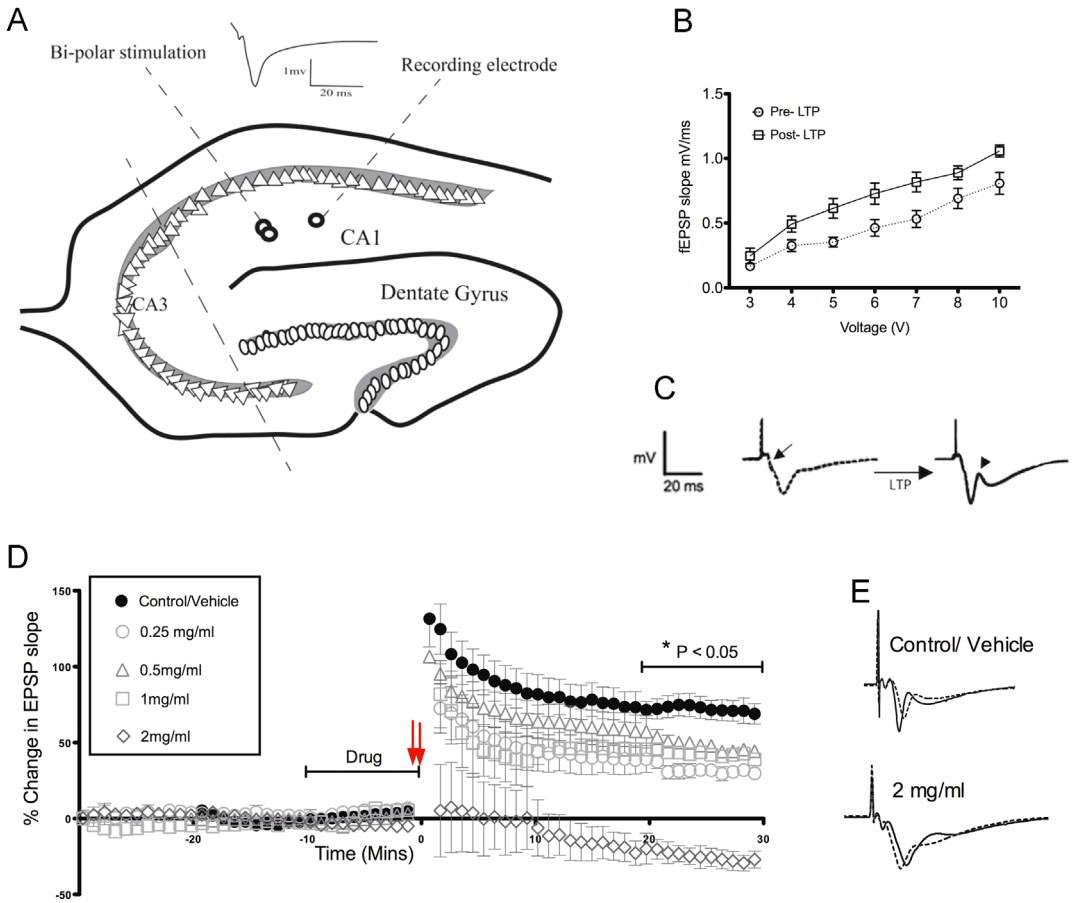

Fig. 1. Induction and modulation of LTP in guinea pig hippocampal slices: (A) position of stimulating and recording electrodes in CA1. The solid line indicates the area removed from the hippocampus; (B) synaptic input (voltage)/output (fEPSP slope) graph. The slopes show an upward-shifted after LTP (squares); (C) fEPSPs before and after the induction of LTP with HFS. The arrow shows the initial slope of the fEPSP, and the arrow head shows a population spike generated by stimulation after the induction of LTP; (D) The effect of 10-min bath application of MBCD (0.25–2 mg/ml) in hippocampal slices. MBCD reduces LTP induced by HFS (arrows) in a dose-dependent manner. Data shown are mean % changes in fEPSP slope ± S.E.M. * indicates that the change in fEPSP represents a significant difference ($P < 0.05$) from control, $^{**}P < 0.01$, $^{***}P < 0.001$ ($n = 6$ per concentration in each experiment); (E) fEPSPs from control slices and from slices treated with MBCD (2 mg/mL). Waveforms shown are from a representative slice experiment and show the last 10 min of baseline (dotted line) and last 10 min of post-HFS recording (solid line).