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Research Report

Bi-lateral changes to hippocampal cholesterol levels during epileptogenesis and in chronic epilepsy following focal-onset *status epilepticus* in mice

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ABSTRACT

Brain cholesterol homeostasis has been shown to be disrupted in neurodegenerative conditions such as Alzheimer's and Huntington's diseases. Investigations in animal models of seizure-induced brain injury suggest that brain cholesterol levels are altered by prolonged seizures (*status epilepticus*) and are a feature of the pathophysiology of temporal lobe epilepsy. The present study measured hippocampal sterol levels in a model of unilateral hippocampal injury triggered by focal-onset *status epilepticus*, and in chronically epileptic mice. *Status epilepticus* was induced by intra-amygdala microinjection of kainic acid and ipsilateral and contralateral hippocampus analyzed. No significant changes were found for ipsilateral or contralateral hippocampal levels of desmosterol or lathosterol at any time after SE as measured by gas chromatography–mass spectrometry. 24S-hydroxycholesterol and cholesterol levels were unchanged up to 24 h after *status epilepticus* but were decreased in the ipsilateral hippocampus during early epileptogenesis and in chronically epileptic mice. Levels of cholesterol were also reduced in the contralateral hippocampus during epileptogenesis and in chronic epileptic mice. Treatment of mice with the anti-inflammatory cholesterol synthesis inhibitor lovastatin did not alter seizures during *status epilepticus* or seizure-induced neuronal death. Thus, changes to hippocampal cholesterol homeostasis predominantly begin during epileptogenesis, occur bi-laterally even when the initial precipitating injury is unilateral, and continue into the chronic epileptic period.

1. Introduction

Cholesterol is a key component of the brain. This is underscored by the fact that the brain is the most cholesterol-rich organ in the body and contains 25–30% of total body cholesterol content. It is a major structural component of plasma membranes and myelin, where it is critical for rapid conduction of nerve transmission. Cholesterol has also been directly

Abbreviations: EEG, electroencephalographic; FJB, Fluoro-Jade B; KA, kainic acid; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling

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implicated in various neuronal functions, including synaptogenesis and neurotransmitter release (Mauch et al., 2001; Thiele et al., 2000). Indirectly it has a general influence on cerebral biochemistry via conversion into bioactive steroids such as neurosteroids and oxysterols. A noteworthy feature of brain cholesterol is that the brain meets its cholesterol requirements entirely by local synthesis, as cholesterol containing lipoproteins from the periphery are excluded from the brain by the blood–brain barrier (Bjorkhem and Meaney, 2004).

Although the average rate of cholesterol synthesis in the adult brain is relatively low there is a need to remove excess cholesterol from the brain (Bjorkhem et al., 1998). One mechanism for this involves cholesterol carried in apolipoprotein E (*APOE*)–containing lipoproteins which pass through the cerebrospinal fluid and eventually reach the circulation where they presumably integrate into peripheral lipoprotein metabolism (Pitas et al., 1987b). The second, more quantitatively important process is based on conversion of cholesterol into the oxysterol 24S-hydroxycholesterol (24S-OHC). In contrast to cholesterol, 24S-OHC can pass the blood–brain barrier and directly enter the general circulation. As 24S-OHC is ultimately cleared by the liver, this system effectively acts as a brain-initiated pathway of reverse cholesterol transport (Lund et al., 1999). The cytochrome P450 enzyme, cholesterol 24-hydroxylase (*CYP46A1*) mediates this transformation and is therefore a key determinant of the rate of cholesterol removal from the brain (Lund et al., 2003). In humans, CYP46A1 is exclusively expressed in the brain and under normal conditions the enzyme protein is detected only in neurons, in particular in the pyramidal neurons and interneurons of the hippocampus (Lund et al., 1999; Ramirez et al., 2008).

Seizures are a co-morbidity in a variety of diseases associated with pathologic cholesterol metabolism. Whether epilepsy, which is characterized by recurrent spontaneous seizures, is also associated with alterations of cholesterol metabolism and whether these are causally important for either seizure-induced neuronal death or the pathogenesis of hippocampal sclerosis, the most common pathologic lesion in temporal lobe epilepsy (Engel et al., 2011), is unknown. Previous studies reported an increase in cholesterol and certain oxysterols in the hippocampus of rats following kainic acid (KA)-induced seizures (Ong et al., 2003). We recently reported that brief seizures in mice increase the expression of StAR-related lipid transfer domain containing 4 (*Stard4*), which can bind cholesterol and is involved in intracellular cholesterol transport (Hatazaki et al., 2007). A number of studies have also explored the effects of statins on seizure-induced neuronal death (He et al., 2006; Lee et al., 2008; Xie et al., 2011). Statins competitively inhibit the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme-A reductase (*HMGCR*), and are a commonly prescribed as cholesterol-lowering agent. Atorvastatin has been reported to reduce hippocampal neuronal death in rodent models of excitotoxicity in vivo (Lee et al., 2008; Piermartiri et al., 2009) and lovastatin treatment of rats reduced sterol levels and increased neuronal survival after *status epilepticus* (He et al., 2006).

The previous reports of a protective effect of statins in rat models of seizure-induced damage prompted us to investigate if this was also the case in a model of *status epilepticus* in mice in which seizures are focally-evoked by intra-amygdala microinjection of KA (Araki et al., 2002; Mouri et al., 2008). The results of this led us to characterize in detail the immediate and long-term effects on brain sterol homeostasis after *status epilepticus* in this model.

2. Results

2.1. *Intra-amygdala KA-induced status epilepticus results in unilateral hippocampal pathology and spontaneous recurrent seizures*

Focal-onset *status epilepticus* in mice was produced by intra-amygdala microinjection of KA. All mice developed seizures within a few minutes after KA injection and displayed typical behavioral changes including initial immobility, tail extension (Straub-tail) and continuing to clonus, head bobbing and rearing and falling, and occasionally tonic-clonic seizures with loss of posture and jumping. Cortical EEG recordings during seizures detected high amplitude and high frequency discharges (Fig. 1B). Animals were administered lorazepam to curtail seizures and reduce morbidity and mortality 40 min after KA. Examination of tissue sections from mice 24 h after *status epilepticus* revealed neuronal cell death mainly in the CA3 region of the ipsilateral hippocampus (Fig. 1C). Neuronal death was not observed in the contralateral hippocampus (Fig. 1C).

Consistent with previous reports (Mouri et al., 2008), intermittent video and EEG monitoring of mice after *status epilepticus* detected the emergence of spontaneous (i.e. epileptic) after KA injection (Fig. 1D and E). In tissue sections from epileptic mice 14 day after *status epilepticus*, the hippocampus displayed neuronal loss within the ipsilateral CA3 subfield (Fig. 1F). The contralateral hippocampus appeared normal, without any obvious changes in cell density (Fig. 1F). To determine whether there was any ongoing cell death in epileptic mice, sections from mice 14 days after *status epilepticus* were stained for DNA fragmentation characteristic of seizure-induced neuronal death using terminal deoxynucleotidyl dUTP nick end labeling (TUNEL) and the neuronal marker (NeuN). No TUNEL-positive cells were detected in the CA3 region of the ipsilateral hippocampus from mice 14 days after *status epilepticus* (Fig. 1G).

2.2. *Lovastatin treatment does not prevent hippocampal damage caused by intra-amygdala KA-induced status epilepticus*

Statins have been reported to have potent neuroprotective effects against seizure-induced neuronal death in rats (Lee et al., 2008; Ohyama et al., 2006; Rangel et al., 2005). To test if lovastatin treatment has such an effect in the present model, mice were treated for three consecutive days with lovastatin using a dose previously shown to lower brain cholesterol in animal models (Lee et al., 2008). On the fourth day, mice received lovastatin before and after being subjected to KA-induced *status epilepticus*, and then received lovastatin again on the two subsequent days. Brains were collected 72 h after *status epilepticus*. Because previous studies have reported that statins can alter seizure severity (Lee et al., 2008), the EEG was recorded for 40 min after KA injection until administration of the anticonvulsant lorazepam and quantified to define the durations of injury-causing electrographic seizures.