Experimental Neurology 225 (2010) 196–201

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Cerebral blood flow changes during pilocarpine-induced status epilepticus activity in the rat hippocampus

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ARTICLE INFO

Article history:
Received 9 March 2010
Revised 1 June 2010
Accepted 20 June 2010
Available online 25 June 2010

Keywords:
Status epilepticus
Hippocampus
Cerebral blood flow
MRI

ABSTRACT

Introduction: There is a known relationship between convulsive status epilepticus (SE) and hippocampal injury. Although the precise causes of this hippocampal vulnerability remains uncertain, potential mechanisms include excitotoxicity and ischaemia. It has been hypothesised that during the early phase of seizures, cerebral blood flow (CBF) increases in the cortex to meet energy demand, but it is unclear whether these compensatory mechanisms occur in the hippocampus. In this study we investigated CBF changes using perfusion MRI during SE in the pilocarpine rat.
Methods: First, we determined whether SE could be induced under anaesthesia. Two anaesthetic protocols were investigated: isoflurane ($n=6$) and fentanyl/medetomidine ($n=7$). Intrahippocampal EEG electrodes were used to determine seizure activity and reflex behaviours were used to assess anaesthesia. Pilocarpine was administered to induce status epilepticus. For CBF measurements, MRI arterial spin labelling was performed continuously for up to 3 h. Either pilocarpine (375 mg/kg) ($n=7$) for induction of SE or saline ($n=6$) was administered. Diazepam (10 mg/kg) was administered i.p. 90 min after the onset of SE.
Results and discussion: We demonstrated time-dependent significant ($p<0.05$) differences between the CBF responses in the parietal cortex and the hippocampus during SE. This regional response indicates a preferential distribution of flow to certain regions of the brain and may contribute to the selective vulnerability observed in the hippocampus in humans.

Introduction

Convulsive status epilepticus (SE) is defined as a convulsive seizure that persists for 30 min or more (Rona et al., 2005) and has been associated with brain injury especially to the hippocampus (Scott et al., 2006; Choy et al., 2010). The mechanisms underlying this vulnerability of the hippocampus remain unclear. Seizure-induced excitotoxicity has been hypothesised to be the main mechanism that leads to injury, although there is some evidence that regional ischaemia may also be an important factor (Fabene et al., 2007; Siesjo and Wieloch, 1986).

It has been suggested that there are acute augmentations (or increases) changes in cerebral blood flow (CBF) during a seizure (compensation phase), following which there is a reduction in CBF (decompensation phase) (for a review see Lothman, 1990). The compensation phase occurs within the first 30 min of a seizure and is characterised by recruitment of physiological mechanisms such as increased blood flow and alterations in pH and glucose metabolism (Lothman and Collins, 1981; Meldrum and Nilsson, 1976). Subsequently, the brain enters the decompensation phase during which several physiological processes, such as cerebral autoregulation, fail and neuronal death may begin to occur (Meldrum and Nilsson, 1976). Since autoregulation maintains local blood flow to the tissue, it has been hypothesised that a failure may result in local ischaemia that could exacerbate injury (Meldrum and Nilsson, 1976). It is possible that such a local ischaemia is part of the pathophysiological process that underlies the vulnerability of the hippocampus to status epilepticus induced injury.

The ability to non-invasively image cerebral haemodynamics during seizures has been hampered by a lack of methods that facilitate the mapping of CBF with high temporal and spatial resolution, over a wide range of blood flows (Calamante et al., 1999). Arterial spin labelling (ASL) is an MRI technique for measuring CBF that uses magnetically labelled blood water as an endogenous tracer (Thomas et al., 2000). This technique does not have a limitation on the number of repeat measurements in a single study, as it does not

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doi:10.1016/j.expneurol.2010.06.015

require the injection of an exogenous tracer. This makes ASL well suited for the continuous monitoring of the timecourse of CBF over durations of minutes to hours, e.g. to monitor the heterogeneous evolution of tissue perfusion after experimental ischemia and reperfusion in animal models (Lythgoe et al., 2002). Therefore, it is ideal for evaluating the timecourse of cerebral blood flow changes associated with status epilepticus. The images generated with ASL can be converted into CBF maps (ml/100 g/min) and are particularly suited to measuring quantitative regional blood flow changes over the course of an epileptic seizure. Despite these benefits, ASL has yet to be used to investigate seizures directly although it has been used for investigating the pathology following seizures (Choy et al., 2010; Ndode-Ekane et al., 2010).

Only a few studies have imaged the brain during seizure activity with MRI. Magnetic resonance diffusion-weighted imaging (DWI) has been shown to be sensitive we investigate whether to regional changes during seizures and most likely reflects microstructural changes in the tissue (van Eijsden et al., 2004; Engelhorn et al., 2007; Zhong et al., 1995). A recent study used BOLD fMRI to demonstrate that seizure activity in rats was associated with negative BOLD signals in hippocampus, indicating a regional hemodynamic change (Schridde et al., 2008). Perfusion MRI has been used in a previous study, which suggested that there is a relationship between the maximum decrease in perfusion and subsequent neuronal loss using a gadolinium-based contrast agent (Engelhorn et al., 2005). However, these techniques do not allow the characterisation of a timecourse and therefore regional blood flow changes as a function of time during the course of status epilepticus.

In this study, we hypothesise that the brain haemodynamic responses to seizures change over time, with the greatest response occurring during the first 30 min of a seizure. We also hypothesise that the haemodynamic response of the hippocampus to seizures is impaired when compared to that of the cerebral cortex. Therefore, the aim of this study was to use continuous arterial spin labelling (CASL) in the pilocarpine model of status epilepticus in rats to characterise the regional blood flow changes from before the onset of SE to seizure termination.

Methods

All animal care and procedures were carried out in accordance with the UK Animals (Scientific Procedures) 1986 Act. As MR imaging of small animals typically requires the use of anaesthesia, an initial study was performed to determine whether pilocarpine can be used to induce status epilepticus under anaesthesia. Following these initial experiments, we investigated the regional nature of CBF changes during pilocarpine-induced SE using ASL.

Study 1. Pilocarpine-induced SE under anaesthesia

Anaesthesia has been shown to be effective for abolishing seizure activity, and used clinically in seizures refractory to conventional anti-convulsant therapy (Shorvon, 1994). However, it is commonly required in MR imaging of small animals as anaesthesia reduces stress and limits motion, which can lead to artefacts in the acquired images (Lukasik and Gillies, 2003). It was therefore necessary to determine that seizures could be induced with pilocarpine under anaesthesia.

Isoflurane is a commonly used gaseous anaesthetic for MR imaging, due to, in part, its rapid clearance and ease of management for the animal in the MR system (Lukasik and Gillies, 2003), although it is known to be an effective anti-convulsant (Shorvon, 1994). Fentanyl, by itself, has been shown to be an effective method for sedation and subsequent induction of seizures (Engelhorn et al., 2005), but does not meet the requirement for anaesthesia (Flecknell, 2009). However, fentanyl in combination with medetomidine, can provide effective anaesthesia (Flecknell, 2009). Therefore we investigated the ability of pilocarpine to induce SE under these two anaesthetic regimens. Thirteen adult male Sprague–Dawley rats (300–350 g) were randomly divided into two groups and anaesthetised with either isoflurane (1.5% in a 60/40 N_2O:O_2 mix delivered at 1 l/min) ($n=6$) or fentanyl citrate (300 μg/kg, i.p) and medetomidine (300 μg/kg, i.p) ($n=7$). Tungsten wire electrodes (50 μm diameter, Science Products GMBH, Germany) were implanted bilaterally at symmetrical points in the hippocampus for EEG recording (AP, −4 mm from bregma; ML, 2 mm, and DV, 3.2 mm from the neocortex). The hippocampal electrodes were referenced to a ground electrode attached to the tail of the animal. EEG was acquired continuously until the end of the experiment.

Methylscopolamine (1 mg/kg, i.p., Sigma-Aldrich) was injected i.p. to reduce the peripheral cholinergic effects of pilocarpine, followed 15–20 min later by pilocarpine hydrochloride injection (375 mg/kg, i.p., Sigma-Aldrich). If seizure activity was detected on EEG, then 90 min after the onset of continuous seizure activity, diazepam (10 mg/kg, i.p., Phoenix Pharma Ltd, Gloucester, UK) was given to attenuate the seizure. Pilocarpine hydrochloride and methylscopolamine were freshly prepared prior to administration in 0.9% saline.

Reflex behaviour was used to assess the level of anaesthesia. Two reflex behaviours were used to provide an estimation of the depth of anaesthesia required for surgical procedures: paw pinch reflex and corneal reflex (Alves et al., 2009; Green, 1982). The depth of anaesthesia was tested every 10 min using these reflex behaviours to determine whether adequate anaesthesia was maintained throughout the experiment. If the animal retained either reflex during any period of the protocol then, for fentanyl, an additional dose of 100 μg/kg of was given, or for isoflurane, the inspired dose was increased.

The presence of status epilepticus was investigated using EEG which was recorded continuously using a Bioamp differential amplifier interfaced with a Powerlab data acquisition system (AD Instruments, Australia). Signals were filtered between 1 and 120 Hz, a 50-Hz notch filter was used to reduce noise, with a sampling rate of 1024 samples per second. Analysis of electrophysiological data was carried out off-line on a Pentium computer, using Chart software (AD Instruments, Australia).

Study 2. CBF measurements with MRI

19 adult male Sprague–Dawley rats (300–400 g, Charles Rivers) were divided randomly into two groups: pilocarpine ($n=13$ of which 6 were discarded because of movement artefacts in the images) or saline ($n=6$). Once the animals had been prepared for imaging (see below), scopolamine (1 mg/kg) was administered. T_1-weighted images were acquired at the beginning of the experiment for the purpose of CBF quantification. Thereafter ASL was acquired continuously for the duration of the experiment.

The experimental design is outlined in Fig. 1. The first 30 min of MRI were acquired for baseline measurements after which the animals were administered either pilocarpine (375 mg/kg) or saline. Bench experiments (see study 1) indicated that SE began approximately 30 min following pilocarpine administration; therefore in order to obtain data from 2 h of continuous seizure activity the animals were imaged for 150 min before diazepam (10 mg/kg) was given. After diazepam the rats were imaged for up to a further 30 min. The animals were visually assessed for seizure activity following each set of perfusion acquisitions.

Animals were anaesthetised with intraperitoneal injections of fentanyl citrate (300 μg/kg) and medetomidine (300 μg/kg). Additional doses of fentanyl (100 μg/kg) were given if the paw pinch reflex was present in order to maintain anaesthesia. O_2 was delivered continuously via a nose cone at a rate of 1 l/min. Rats were placed on a specially-designed animal holder to minimize motion artifacts. Physiological monitoring included electrocardiography (ECG) recordings and rectal temperature recordings. Temperature was maintained at 37 °C ± 2.