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Research Report

In vivo glutamate decline associated with kainic acid-induced status epilepticus

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ABSTRACT

Neurophysiological, biochemical, and anatomical evidence implicates glutamatergic mechanisms in epileptic seizures. Until recently, however, longitudinal characterization of in vivo glutamate dynamics was not possible. Here, we present data using in vivo magnetic resonance spectroscopy (MRS) optimized for the detection of glutamate to identify changes that evolve following kainic acid (KA)-induced status epilepticus. Wild-type male Wistar rats underwent whole-brain MR imaging and single-voxel MRS on a clinical 3 T scanner equipped with a high-strength insert gradient coil. Scanning took place before and then 3 days, 28–32 days, and 42–50 days after induction of status epilepticus. Analyses compared 5 seizure (Sz), 5 no-seizure (NoSz; received KA but did not exhibit seizures), and 6 control (Con) animals. This longitudinal study demonstrated reduced glutamate levels in vivo in the dorsal hippocampus 3 days and 1 month following status epilepticus in Sz animals compared with Con animals. Additionally, previous results were replicated: in the Sz group, computed T2 was higher in the ventral hippocampus and limbic cortex 3 days after seizure activity compared with baseline but resolved in both regions at the 1 month scan, suggesting a transient edema. Three days following seizure activity, *N*-acetylaspartate (NAA) declined and lactate increased in the dorsal hippocampus of the Sz group compared with the Con and NoSz group; both metabolites approached baseline levels by the third scan. Taken together, these results support the conclusion that seizure activity following KA infusion causes loss of glutamatergic neurons.

1. Introduction

Ample data support a role for glutamate in epilepsy. In vivo microdialysis studies report elevated extracellular glutamate levels before or during seizures in hyperexcitable brain regions in animal models of epilepsy (Liu et al., 1997; Sierra-Paredes et al., 1998; but see Tanaka et al., 1996; Wade et al., 1987) and in patients with medial temporal lobe epilepsy (Carlson et al., 1992; During and Spencer, 1993; Wilson et al., 1996). A sustained elevation in basal release of glutamate (Zhang et al., 1991), in addition to initiating and maintaining epileptic seizures, may be excitotoxic and contribute to cell death (e.g.,

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Magloczky and Freund, 1993; Meldrum, 1993). In the interictal stage, in vitro studies report low glutamate content in hippocampal tissue from both animals (Koyama, 1972) and humans (Peeling and Sutherland, 1993) potentially reflecting the loss of glutamatergic neurons of the hippocampus. Recent in vitro magnetic resonance spectroscopy (MRS) studies demonstrate a reduction in the amount of ^{13}C labeling of glutamate in the hippocampal formation of systemically treated kainic acid (KA) rats (Alvestad et al., 2008) and biopsied hippocampal tissue from patients with medial temporal lobe epilepsy (Petroff et al., 2002). However, our understanding of the role of altered glutamate homeostasis in epilepsy is incomplete, especially in vivo, and techniques such as in vivo microdialysis have limited applicability to longitudinal studies (Plock and Kloft, 2005).

Temporal lobe epilepsy can be modeled in rodents by administration of KA (Williams et al., 2007). As an excitatory amino acid agonist (Ferkany et al., 1982), KA can provoke prolonged seizures (i.e., status epilepticus), characterized by salivation, rearing, bilateral upper extremity clonus, and falling (Lothman and Collins, 1981), and set in motion a process of epileptogenesis that may lead to spontaneous seizures (Williams et al., 2007). Postmortem histological evaluation demonstrates a specific pattern of neuronal loss largely restricted to cells of the hippocampus, amygdala, and related parts of the thalamus and cortex (e.g., piriform and entorhinal cortices; Nadler and Cuthbertson, 1980; Schwob et al., 1980; Sperk et al., 1983; Sperk, 1994). This specific regional profile of brain damage in rodents following KA-induced status epilepticus resembles Ammon's horn sclerosis (Sagar and Oxbury, 1987). Neuronal loss as a consequence of KA-induced status epilepticus involves, at last in part, an apoptotic mechanism dependent on activation of caspase-3 protease activity (Faherty et al., 1999; Kondratyev and Gale, 2000, 2004; Puig and Ferrer, 2002; Tokuhara et al., 2007).

In vivo studies using magnetic resonance imaging (MRI) protocols sensitive to mobile water protons (e.g., T2-weighted images or computed T2 maps) have enabled tracking of the evolution of brain changes associated with KA-induced seizures. T2-weighted images typically detect hyperintensities at 24 h (e.g., TE=272 ms; Ebisu et al., 1996) that persist for 3 days after systemic KA injection (Ebisu et al., 1994) and for 10 days following local intracerebral KA injections (e.g., Bouilleret et al., 2000; Luna-Medina et al., 2007; Tanaka et al., 1993). Postmortem analyses suggest that regions of hyperintense T2-weighted signals in the acute period following seizure activity reflect edema (e.g., Hantraye et al., 1992; Tanaka et al., 1993).

Along with gross morphology and tissue quality measurements from structural MRI, chemical constituents of tissue are detectable with MRS (cf., Adalsteinsson et al., 2002; Zahr et al., 2009). N-Acetylaspartate (NAA), a presumed neuronal marker, is lower in the rat hippocampus (Najm et al., 1997, 1998) and piriform cortex (Ebisu et al., 1996) 24 h to at least 7 days (Najm et al., 1997) after systemically administered KA; NAA reductions persist for up to 84 days following local intracerebral stereotaxic injections of KA (Luna-Medina et al., 2007; Tokumitsu et al., 1997). KA administration is also associated with an increase in hippocampal lactate that occurs within 3 h of seizure induction (Meric et al., 1994) and persists for 7 days after systemic injection (Najm et al., 1997, 1998) and upwards of 9 days after local hippocampal injection of KA (Luna-Medina et al., 2007).

The present in vivo longitudinal study was undertaken to replicate previous MRI and MRS results and to extend earlier work by quantifying glutamate levels at baseline and as long as 1 month following KA-induced status epilepticus. We hypothesized that MRI would detect a transient increase in computed T2, indicative of edema in response to systemic KA-induced status epilepticus, and that structural evidence for neuronal loss in limbic structures would be paralleled by ventricular enlargement. We also predicted that NAA levels would decrease and lactate levels would increase in the dorsal hippocampus as a result of KA-induced seizure activity. Notably, based on recent in vitro studies using ^{13}C labeled glucose, we expected levels of glutamate to decline in the dorsal hippocampus in the interictal period following status epilepticus. Confirmation of previous results (reduced NAA and increased T2 and lactate) supports the novel in vivo finding of decreased glutamate following KA-induced status epilepticus. Radiological results were verified with postmortem immunohistochemical staining for caspase-3.

2. Results

Between-group analyses were conducted on computed T2 data from scans 1 and 2 and on MRS data from scans 1, 2, and 3. Only the seizure (Sz) group was scanned 4 times and formed the basis for within-subject analyses of ventricular volume (Table 1). Body weight data for the control (Con) and no seizure (NoSz) groups is absent at the 42- to 50-day time point since

Table 1 – Scan schedule and weights (g) ± SE.

	N	Scan 1 Baseline	Scan 2 3 days after KA	Scan 3 28–32 days after KA	Scan 4 42–50 days after KA
Control	6	391.8 ± 6.8 [a,b]	397.2 ± 7.5 [a,b]	456.7 ± 9.4 [b]	NA
KA-no seizure	5	383.4 ± 6.3 [a,b]	374.6 ± 7.4 [a,b]	441.6 ± 6.0 [b]	NA
KA-seizure	5	396.4 ± 6.7 [a,b,c]	396.6 ± 19.6 [a,b,c]	452.8 ± 17.5 [b,c]	473.8 ± 18.4 [b,c]

NA = not required.
[a] T2 data analyzed.
[b] MRS data analyzed.
[c] Ventricular volume analyzed.