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Electrographic seizures are significantly reduced by in vivo inhibition of neuronal uptake of extracellular glutamine in rat hippocampus

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Summary Rats were given unilateral kainate injection into hippocampal CA3 region, and the effect of chronic electrographic seizures on extracellular glutamine (GLN_{ECF}) was examined in those with low and steady levels of extracellular glutamate (GLU_{ECF}). GLN_{ECF}, collected by microdialysis in awake rats for 5 h, decreased to $62 \pm 4.4\%$ of the initial concentration ($n = 6$). This change correlated with the frequency and magnitude of seizure activity, and occurred in the ipsilateral but not in contralateral hippocampus, nor in kainate-injected rats that did not undergo seizure ($n = 6$). Hippocampal intracellular GLN did not differ between the Seizure and No-Seizure Groups. These results suggested an intriguing possibility that seizure-induced decrease of GLN_{ECF} reflects not decreased GLN efflux into the extracellular fluid, but increased uptake into neurons. To examine this possibility, neuronal uptake of GLN_{ECF} was inhibited in vivo by intrahippocampal perfusion of 2-(methylamino)isobutyrate, a competitive and reversible inhibitor of the sodium-coupled neutral amino acid transporter (SNAT) subtypes 1 and 2, as demonstrated by 1.8 ± 0.17 fold elevation of GLN_{ECF} ($n = 7$). The frequency of electrographic seizures during uptake inhibition was reduced to $35 \pm 7\%$ ($n = 7$) of the frequency in pre-perfusion period, and returned to $88 \pm 9\%$ in the post-perfusion period. These novel in vivo results strongly suggest that, in this well-established animal model of temporal-lobe epilepsy, the observed seizure-induced decrease of GLN_{ECF} reflects its increased uptake into neurons to sustain enhanced glutamatergic epileptiform activity, thereby demonstrating a possible new target for anti-seizure therapies.

Abbreviations: EAAT, excitatory amino acid transporter; ECF, extracellular fluid; GABA, γ-aminobutyric acid; GLN, glutamine; GLU, glutamate; KA, kainic acid; MeAIB, 2-(methylamino)isobutyrate; SNAT, sodium-coupled neutral amino acid transporter; TAU, taurine.

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Introduction

Temporal-lobe epilepsy, which accounts for 40% of epilepsy, is associated with an epileptic focus in the hippocampus resulting from local brain injury. The mechanism of the onset of chronic recurrent seizures, following a latent period, is a subject of intense investigation (reviewed by (Bradford, 1995; Jefferys, 2010; Morimoto et al., 2004). Among numerous animal models, the chronic kainate-induced rodent model resembles most closely the EEG and biochemical abnormalities of temporal-lobe epilepsy (Mathern et al., 1993; Riban et al., 2002; Tanaka et al., 1992). The rat develops chronic recurrent seizures between 3 and 90 days after unilateral intrahippocampal injection of kainate (KA) (Bragin et al., 1999, 2005), which is an agonist of the KA receptor of the ionotropic glutamate receptor family (Vincent and Mulle, 2009).

Our recent study using this model showed that chronic recurrent seizures with no or mild behavioral components (electrographic seizures) caused not only elevation of the excitatory neurotransmitter glutamate in the extracellular fluid (GLU_{ECF}), but also a significant decrease in the concentration of its precursor glutamine in the extracellular fluid (GLN_{ECF}) (Kanamori and Ross, 2011). Decrease of GLN_{ECF} associated with chronic epileptiform activity was a novel finding that had not been reported previously.

The physiological basis of our study is illustrated in Fig. 1 which shows schematically the major metabolic pathways of GLU, GLN and γ-aminobutyric acid (GABA) and their transport pathways between synaptic vesicles, extracellular fluid (ECF), glia and the neuron, according to a suggested model of GLN/GLU/GABA cycle (Erecinska and Silver, 1990; Hertz, 1979; Shank and Aprison, 1981) (see section ''Role of GLN_{ECF} in sustaining epileptiform activity in vivo'' for discussion of an alternative model).

The questions that we address in the present paper are the following:

1. Does the decrease in GLN_{ECF}, associated with seizure, also occur in those KA rats that exhibit no detectable change in GLU_{ECF}?
2. If so, does the change in GLN_{ECF} correlate with the frequency and magnitude of chronic electrographic seizures?
3. If a correlation is found, does the decrease in GLN_{ECF} reflect decreased efflux of glial GLN to ECF or its increased uptake into neurons (Fig. 1)?
4. If the latter is likely, how does inhibition of neuronal uptake of GLN_{ECF} in vivo affect the frequency of electrographic seizures?

To address questions #1 and #2, we examined GLN_{ECF} in those KA rats that exhibit no significant change in GLU_{ECF}. Specifically, we examined the frequency and magnitude of EEG seizures and the time-course of GLN_{ECF} in (a) the ipsilateral (KA-injected) hippocampus of seizure-exhibiting vs seizure-free rats, and also (b) in the seizure-prone ipsilateral vs seizure-free contralateral hippocampus of the same rat. To address question #3, we measured the concentrations of hippocampal intracellular GLN (as well as of GLU and GABA) in seizure-exhibiting vs seizure-free KA rats. To address question #4, we took a novel approach of inhibiting in vivo the SNAT1/SNAT2 mediated neuronal uptake of GLN_{ECF} (Fig. 1) by intrahippocampal perfusion of 2-(methylamino)isobutyrate (MeAIB), a competitive, non-metabolizable and reversible inhibitor of System A transporter (Christensen, 1990; Christensen et al., 1965), and examined the effect of this inhibition on the frequency of electrographic seizures.

We report here the results of these studies which strongly suggest that (a) the observed GLN_{ECF} decrease in response to epileptiform activity reflects increased neuronal uptake, and (b) uptake inhibition significantly reduces the frequency of electrographic seizures in vivo. Implications of the results for a major role of GLN in sustaining excitatory neurotransmission during enhanced seizure activity are discussed.

Material and methods

Kainic acid injection

Adult male Wistar rats (240—340 g) were anesthetized with pentobarbital (40 mg/kg wt) and placed on a stereotaxic instrument. Kainic acid (KA) was injected unilaterally into CA3 region of the right hippocampus at AP = −5.6 mm, L = +4.5 mm and V = 5.5 mm (Paxinos and Watson, 1997). Sodium kainate (Sigma—Aldrich, St. Louis, MO, USA), dissolved in 0.1 M phosphate buffer, was injected with a 0.5 μL syringe at a dose of 0.4 μg/0.2 μL for a 325 g rat and adjusted according to body weight, as described previously (Kanamori and Ross, 2011). The rat, which awoke from anesthesia within 1 h, was continuously monitored for behavioral seizures for 6 h after injection (acute phase).

Chronic-phase procedures

During the chronic phase at 37—46 days after KA injection, we implanted EEG recording and grounding electrodes (Plastics One, Roanoke, VA, USA) and microdialysis guide cannula with a stylet (BioAnalytical systems, West Lafayette, IN, USA), as described previously (Kanamori and Ross, 2011). Three experimental protocols, with objectives described in section ''Overview of experimental design'', were used in the present study. The surgical and experimental procedures for each protocol are described below.

EEG/microdialysis (Experiment I)

In Experiment I, EEG recordings were taken bilaterally with microdialysis only in the ipsilateral hippocampus, in overnight-fasted rats. The EEG electrode was implanted at AP = −5.6 mm, L = −4.5 mm and V = 5.5 mm in the contralateral hippocampus. In the ipsilateral hippocampus, the coordinates were AP = −5.6 mm, L = 4.5 mm and V = 5.5 mm for the electrode, and for the guide cannula attached to the electrode, V = 3.5 mm. This places the electrode tip in CA3 region and the tip of the microdialysis guide cannula, just above the CA1 region (Paxinos and Watson, 1997). For preliminary bilateral EEG recording from an awake rat one week after the surgery, the electrode contacts on the skull were connected through a commutator to an amplifier