Epilepsy Research (2015) 114, 32—46

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

Disinhibition reduces extracellular glutamine and elevates extracellular glutamate in rat hippocampus in vivo

Keiko Kanamori*

Huntington Medical Research Institutes, 660 South Fair Oaks Avenue, Pasadena, CA 91105, USA

Received 8 October 2014; received in revised form 14 January 2015; accepted 16 March 2015
Available online 23 March 2015

KEYWORDS
Extracellular glutamine;
Extracellular glutamate;
Disinhibition;
Rat hippocampus;
$GABA_A$/$GABA_B$ receptor antagonists;
Epileptiform discharge

Summary Disinhibition was induced in the hippocampal CA1/CA3 region of normal adult rats by unilateral perfusion of the $GABA_AR$ antagonist, 4-[6-imino-3-(4-methoxyphenyl)pyridazin-1-yl] butanoic acid hydrobromide (gabazine), or a $GABA_BR$ antagonist, *p*-(3-aminopropyl)-*p*-diethoxymethyl-phosphinic acid (CGP 35348), through a microdialysis probe. Effects of disinhibition on EEG recordings and the concentrations of extracellular glutamate (GLU_{ECF}), the major excitatory neurotransmitter, and of extracellular glutamine (GLN_{ECF}), its precursor, were examined bilaterally in freely behaving rats. Unilateral perfusion of 10 μM gabazine in artificial CSF of normal electrolyte composition for 34 min induced epileptiform discharges which represent synchronized glutamatergic population bursts, not only in the gabazine-perfused ipsilateral hippocampus, but also in the aCSF-perfused contralateral hippocampus. The concentration of GLU_{ECF} remained unchanged, but the concentration of its precursor, GLN_{ECF}, decreased to $73 \pm 4\%$ ($n = 5$) of the baseline during frequent epileptiform discharges, not only in the ipsilateral, but also in the contralateral hippocampus, where the change can be attributed to recurrent epileptiform discharges per se, with recovery to 95% of baseline when epileptiform discharges diminished.

The blockade of $GABA_BR$, by CGP 35348 perfusion in the ipsilateral hippocampus for 30 min, induced bilateral Na^+ spikes in extracellular recording. These can reasonably be attributed to somatic and dendritic action potentials and are indicative of synchronized excitatory activity. This disinhibition induced, in both hippocampi, (a) transient 1.6—2.4-fold elevation of GLU_{ECF} which correlated with the number of Na^+ spike cluster events and (b) concomitant reduction of GLN_{ECF} to ~70%.

Intracellular GLN concentration was measured in the hippocampal CA1/CA3 region sampled by microdialysis in separate groups of rats by snap-freezing the brain after 25 min of gabazine perfusion or 20 min of CGP perfusion when *extracellular* GLN (GLN_{ECF}) was 60—70% of the pre-perfusion level. These intracellular GLN concentrations in the disinhibited hippocampi showed

Abbreviations: CGP, CGP 35348; ECF, extracellular fluid; $GABA_AR$, $GABA_A$ receptor; $GABA_BR$, $GABA_B$ receptor; GLN_{ECF}, extracellular glutamine; GLU_{ECF}, extracellular glutamate; HC, hippocampus.

* Tel.: +1 626 397 5843; fax: +1 6263975848.
E-mail address: kkanamori@hmri.org

http://dx.doi.org/10.1016/j.eplepsyres.2015.03.009

no statistically significant difference from the untreated control. This result strongly suggests that the observed decrease of GLN_{ECF} is not due to reduced glutamine synthesis or decrease in the rate of efflux of GLN to ECF. This strengthens the likelihood that reduced GLN_{ECF} reflects increased GLN uptake into neurons to sustain enhanced GLU flux during excitatory population bursts in disinhibited hippocampus. The results are consistent with the emerging concept that neuronal uptake of GLN_{ECF} plays a major role in sustaining epileptiform activities in the kainate-induced model of temporal-lobe epilepsy.

Introduction

An imbalance between excitatory and inhibitory neurotransmission is a widely accepted candidate mechanism for epileptogenesis (reviewed by Trevelyan and Schevon, 2013). Epileptic seizures, according to well-supported theory, are caused by glutamate excitotoxicity when the major excitatory neurotransmitter glutamate is released into the extracellular fluid (ECF) faster than it is taken up into glia, leading to overstimulation of glutamate receptors (Bradford, 1995; During and Spencer, 1993). The CA3 region of the hippocampus (HC), which is highly populated with pyramidal glutamatergic neurons with recurrent networks, is especially susceptible to synchronized excitatory population bursts that result in glutamate excitotoxicity. CA3 pyramidal neurons innervate the dendrites of CA1 pyramidal cells. Normally, excitation of these glutamatergic neurons is under control of GABAergic inhibitory interneurons (Chrobak and Buzsáki, 1996). The axon terminals of GABAergic neurons target and inhibit the somatodendritic region and the axon initial segment of the pyramidal cells (Lovett-Barron et al., 2012; Ylinen et al., 1995). GABA, acting on ionotropic $GABA_A$ receptor on glutamatergic neurons, mediates fast inhibitory post-synaptic potentials via Cl^- influx, which results in hyperpolarization of the postsynaptic pyramidal cells and an increase in the threshold for firing. GABA can also act on metabotropic $GABA_B$ receptors. $GABA_B$ receptor activation in the hippocampal CA1/CA3 synaptic circuits is predominantly inhibitory because of the inhibition of glutamate release via presynaptic heteroreceptors (Biermann et al., 2010). Thus, pyramidal neurons are subject to two antagonistic polarizations; dendritic excitation from glutamatergic neurons and somatic/perisomatic inhibition from GABAergic interneurons. When inhibitory control is weakened or lost (disinhibition), massive depolarization of the target pyramidal neurons occurs, resulting in epileptiform discharges.

Our previous studies on the metabolic and pathophysiological bases of glutamate excitotoxicity showed that GLN_{ECF}, which upon take into neurons, serves as the precursor of the metabolic and neurotransmitter pools of GLU, is significantly reduced in response to electrographic seizures in kainate-induced rat model of temporal lobe epilepsy (Kanamori and Ross, 2011). This novel finding raised an intriguing possibility that neuronal uptake of GLN_{ECF} is accelerated during epileptic seizures to replenish the neurotransmitter pool of glutamate. To examine this hypothesis, the present study investigates the effects of glutamatergic population bursts induced by disinhibition on GLU_{ECF} and GLN_{ECF}. Disinhibition was induced in normal adult rat hippocampus (HC) by unilateral perfusion of (a) the ionotropic $GABA_AR$ antagonist, gabazine (Mienville and Vicini, 1987), or (b) the metabotropic $GABA_BR$ antagonist CGP 35348 (Olpe et al., 1990). $GABA_A$ antagonism induced epileptiform discharges and $GABA_B$ antagonism induced complex Na^+ spike clusters, not only in the treated ipsilateral, but also in the contralateral HC by transmission of the neuronal activity through the commissural fibers. Hence, in the aCSF-perfused contralateral HC, changes in GLU_{ECF} and GLN_{ECF} can reasonably be attributed to the occurrence of glutamatergic population bursts per se, without additional complex effects of perfusion as in the ipsilateral HC. The results show that GLN_{ECF} is significantly reduced in both disinhibition paradigms, while GLU_{ECF} is transiently elevated with $GABA_BR$ blockade. Collectively, these results strongly suggest an important role for GLN_{ECF} in sustaining high flux of neurotransmitter glutamate during excitatory population bursts in disinhibited hippocampus.

Material and methods

Implantation of EEG electrodes and microdialysis guide cannula

All studies were approved by the HMRI Institutional Animal Care and Use Committee in conformance with the NIH Guide for the Care and Use of Laboratory Animals. Adult male Wistar rats (275—400 g) were anesthetized with ketamine/xylazine (100/5.2 mg/kg wt) and placed on stereotaxic instrument. The EEG recording electrode consisted of a pair of stainless steel wires (0.125 mm in diameter, 20 mm in length and tips 0.5 mm apart) that were terminated with a pair of sockets (Plastics One, Roanoke, VA, USA). The grounding electrode was single wire of the same dimension. The recording electrode was attached (with Loctite 401) to a microdialysis guide cannula fitted with a stylet (Bioanalytical Systems, West Lafayette, IN, USA), so that the electrode tips were 1.7 mm below the end of the guide cannula. This EEG electrode/microdialysis guide cannula complex was implanted bilaterally at coordinates of $AP = -5.6$ mm and $L = \pm 4.4$ mm, and $V = 5.4$ mm for the electrode and 3.7 mm for the guide cannula. As shown in Fig. 1 inset, this places the electrode tips in the CA3 region, and the end of the microdialysis guide cannula in the CA1 region, within ~0.5 mm of the dentate gyrus. The grounding electrode was fixed to the parietal bone with an anchor screw. The sockets (one from the grounding electrode and two pairs from the recording electrodes) were inserted into the bottom contacts of a 6-pin