Neurobiology of Disease 82 (2015) 213–225

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Hypoglycemia-induced alterations in hippocampal intrinsic rhythms: Decreased inhibition, increased excitation, seizures and spreading depression

C.M. Florez [a,b], V. Lukankin [a], S. Sugumar [a], R. McGinn [a], Z.J. Zhang [a], L. Zhang [a], P.L. Carlen [a,b,*]

[a] Departments of Medicine (Neurology) and Physiology, University of Toronto, Toronto, Canada
[b] Division of Fundamental Neurobiology, TWRI, UHN, Toronto, Canada

ARTICLE INFO

Article history:
Received 9 November 2014
Revised 9 June 2015
Accepted 12 June 2015
Available online 18 June 2015

Keywords:
Hippocampus
In-vitro
Seizure
Spreading depression
Hypoglycemia
Neuroglycopenia
In-vitro brain rhythms
Brain oscillations

ABSTRACT

Seizures are the most common clinical presentation of severe hypoglycemia, usually as a side effect of insulin treatment for juvenile onset type 1 diabetes mellitus and advanced type 2 diabetes. We used the mouse thick hippocampal slice preparation to study the pathophysiology of hypoglycemia-induced seizures and the effects of severe glucose depletion on the isolated hippocampal rhythms from the CA3 circuitry.

Methods and results: Dropping the glucose perfusate concentration from the standard 10 mM to 1 mM produced epileptiform activity in 14/16 of the slices. Seizure-like events (SLEs) originated in the CA3 region and then spread into the CA1 region. Following the SLE, a spreading-depression (SD)-like event occurred (12/16 slices) with irreversible synaptic failure in the CA1 region (8/12 slices). CA3 SD-like events followed ~30 s after the SD-like event in the CA1 region. Less commonly, SD-like events originated in the CA3 region (4/12). Additionally, prior to the onset of the SLE in the CA3 area, there was decreased GABA correlated baseline SPW activity (bSPW), while there was increased large-amplitude sharp wave (LASW) activity, thought to originate from synchronous pyramidal cell firing. CA3 pyramidal cells displayed progressive tonic depolarization prior to the seizure which was resistant to synaptic transmission blockade. The initiation of hypoglycemic seizures and SD was prevented by AMPA/kainate or NMDA receptor blockade.

Conclusions: Severe glucose depletion induces rapid changes initiated in the intrinsic CA3 rhythms of the hippocampus including depressed inhibition and enhanced excitation, which may underlie the mechanisms of seizure generation and delayed spreading depression.

Introduction

Hypoglycemia is a leading cause of neurological sequelae in infants suffering from severe metabolic or infectious disorders (Montassir et al., 2009), and the most common side effect of insulin treatment in individuals with type 1 diabetes (Cryer, 2010) and insulin-dependent type 2 diabetes (Avila-Fematt and Montana-Alvarez, 2010). Seizures are the most common clinical presentation (>80%) of severe neonatal hypoglycemia, with glucose levels usually below 2 mM (Burns et al., 2008). EEG recordings in humans and in animal models of hypoglycemia show a pattern of decreased alpha and beta activity, large delta waves (Auer et al., 1984), spike and bursting activity (Gibbs and Murray, 1954), seizures, and finally isoelectricity (Lewis et al., 1974).

The anterior temporal lobe and hippocampus are among the most epileptogenic areas of the brain and have been implicated as possible trigger zones for hypoglycemic seizures (Baloyannis et al., 1987; Gibbs and Murray, 1954; Lapenta et al., 2010), although in vivo EEG recordings have not clearly established the areas of seizure origin (Del Campo et al., 2009; Gibbs and Murray, 1954; Velisek et al., 2008). It is clear that severe hypoglycemia can lead to neuronal death predominantly in the hippocampus and in the superficial layers (layers II–III) of the occipital and parietal cortices, and striatum (Auer, 2004; Auer et al., 1985a; Auer et al., 1985b; Bree et al., 2009). This pattern of damage seems to be related to the direct effects of massive release of aspartate and glutamate (Auer, 2004). After severe hypoglycemia, there is cellular degeneration in the dentate gyrus (DG) and CA1, with less involvement of the CA3 region (Auer, 2004; Shin et al., 2010). The reasons for this difference in regional cellular survival in the hippocampus are not well understood. Functionally, in vitro hypoglycemic experiments showed that the CA1 region is susceptible to synaptic failure (Abdelmalik et al., 2007; Crepel et al., 1992; Fan et al., 1988; Sadgrove et al., 2007), an effect exacerbated by the presence of seizures (Abdelmalik et al., 2007).

Abbreviations: bSPW, baseline sharp wave; LASW, large amplitude sharp wave; SLE, seizure-like event; SD, spreading depression-like event.

* Corresponding author at: Toronto Western Hospital, Krembil Discovery Tower, 7th Floor Room 7KD402, 60 Leonard Avenue, Toronto, Ontario M5T 2S8, Canada.
E-mail address: carlen@uhnresearch.ca (P.L. Carlen).

Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.06.005
0969-9961/

In a previous report, our group described a model of hypoglycemic seizures in the intact hippocampal preparation from mice ≤ 13 days/old (Abdelmalik et al., 2007). In that work, changing the perfused glucose concentration from 15 mM to 4 mM induced seizure-like events (SLEs) recorded in CA1 in 73% (11/15) of the preparations. However, the onset zone and the cellular mechanisms associated with the hypoglycemia-induced seizure like events (SLE) within the hippocampus were not clearly established. In this work, we used "thick" hippocampal slices from more mature animals aged 14–21 days to establish the putative area of seizure origin in the hippocampal formation and the cellular effects of low glucose perfusion in CA3 pyramidal cells.

Additionally, we describe the effects of hypoglycemia on two intrinsic rhythms observed in the thick hippocampal slice; baseline sharp-waves (bSPW) which are strongly correlated with $GABA_A$ inhibitory postsynaptic potentials (IPSPs) on CA3 pyramidal cells (Ellender et al., 2010; Hajos et al., 2013; Maier et al., 2003; Wong et al., 2005), and the large amplitude sharp waves (LASW) (El-Hayek et al., 2013; Wu et al., 2002, 2005b, 2009), a designation given to >300 μV SPW-like events associated with spontaneous increased excitatory neurotransmission and pyramidal cell firing observed spontaneously, or induced by tetanic stimulation in mouse isolated thick hippocampal slices. Here, we show that hypoglycemia depressed bSPW and enhanced LASW along with a progressive neuronal membrane depolarization and glutamate receptor activation, phenomena associated with seizure initiation in the CA3 region, often followed by spreading depression in the CA1 region.

Materials and methods

Tissue preparation

C57BL6 male mice postnatal days 14–21 from Charles River Breeding Farm (Montreal, Quebec, Canada) were used. They were housed with one to six other littermates and a nursing mother in a vivarium with a 12 h light/dark cycle at a constant 23 °C. All the procedures presented in this work were approved by the local animal care committee and followed the Canadian Council on Animal Care guidelines. From these animals, thick slices were obtained as previously described. Briefly, under general anesthesia with pentobarbital (70 mg/kg), the mice were perfused intracardially with a neuroprotective solution consisting of sucrose 248 mM, KCl 2 mM, $MgSO_4$ 3 mM, $CaCl_2$ 1 mM, $NaHCO_3$ 26 mM, NaH_2PO_4 1.25 mM, and D-glucose 10 mM followed by fast decapitation and brain removal. The brain was hemisectioned and placed into cold (~4 °C) dissecting solution containing 1 mM of kynurenic acid for 4–5 min, followed by the brain stem and thalamic tissue removal, then glued and sliced to 800 μm or 500 μm sagittal to the hippocampal axis, using a semiautomatic vibrotome (Leica VT1200), the surrounding neocortex and entorhinal cortex were removed, and finally dentate gyrus separated from CA1 and CA3 using a glass probe (Wu et al., 2005b). The recovered slices were placed into a storage chamber containing artificial cerebrospinal fluid (ACSF) made of NaCl 123 mM, KCl 3.5 mM, $CaCl_2$ 1.5 mM, $MgSO_4$ 1.6 mM, sodium bicarbonate 25 mM, NaH_2PO_4 1.2 mM, D-glucose 10 mM, osmolarity of 298 ± 7, with a pH of 7.37 ± 0.05 after bubbling with a mixture of 95% O_2 and 5% CO_2, at 34 °C for 30 min. We allowed passive cooling to room temperature (21 °C) and recovery for at least one-half hour prior to starting the recording protocol.

Electrophysiology

Recordings were carried out in a modified Haas interface chamber for extracellular field recordings as previously described by Wu et al. (2002), and a custom made submersion chamber for patch clamp experiments. Both systems use a bridge consisting of a metallic ring and a mesh on top to allow a raised placement of the tissue from the floor of the chamber, permitting the perfusion of solution (flow rate 10–15 cm^3/min) above and below the brain slice, in order to maximize the oxygen diffusion into the preparation, thereby improving the probability of observing spontaneous hippocampal rhythmic activities (Hajos et al., 2009; Wu et al., 2002). Under these conditions, only slices with a stable evoked response of ≥0.5 mV and the presence of afterdischarges after a maximum of two 1 s stimuli at 100 Hz using 70% of the amperage required to obtain the maximum amplitude of field excitatory post-synaptic potentials (fEPSPs) in CA3 and CA1 were used for the challenge protocol. To evaluate the effects of severe hypoglycemia on the CA3 bSPWs while avoiding the development of seizure-like events (SLEs), 500 μm brain slices were used. In our experience, their more limited circuitry seems to fail in generating hypoglycemia induced SLEs in submerged perfusion chambers.

Dual extracellular field recordings of either CA3 and CA1 pyramidal layers or CA3 pyramidal layer and stratum radiatum were performed using glass micropipettes (World Precision Instruments, Inc., Sarasota, FL), 1 μm tip diameter (1–2 MΩ resistance), filled with 150 NaCl solution (Abdelmalik et al., 2007). The signals were amplified by an Axopatch 200B or a MultiClamp 700B amplifier (Axon Instruments), with sampling rates of 10 kHz, low-pass filtered to 5 kHz. Field potentials were evoked by constant current stimulation of mossy fibers using a custom made bipolar stimulating electrode (0.1–0.15 ms, 40–150 μA, 1–2 per minute), eliciting maximum evoked field excitatory post-synaptic potentials (fEPSPs).

Somatic whole cell recordings under current or voltage clamp mode were obtained using patch pipettes containing (in mM) 10 NaCl, 135 K-gluconate, 1 $MgCl_2$, 10 NaHepes, 0.3 NaGTP, 2 NaATP, pH 7.25, slightly modified from McBain (1994), with a final resistance in the bath ranging from 5–7 MΩ. Only cells with a resting membrane potential (RMP) more negative than −50 mV, stable access resistance (<20 MΩ), and input resistance >100 MΩ were used for subsequent analyses. Continuous monitoring of access resistance was performed before and during the hypoglycemic challenge. Spontaneous and evoked EPSPs and IPSPs were obtained at the RMP and quantified for frequency and amplitude changes during the different experimental conditions.

Hypoglycemic challenge protocol

The challenge protocol consisted of a baseline of 15–30 min ACSF (D-glucose 10 mM) perfusion, followed by up to 30 min of severe hypoglycemia (D-glucose 1 mM), and in a subgroup of slices a subsequent 20–30 min of glucose reperfusion. In a different group of slices, rescue glucose reperfusion was started immediately if seizure-like events (SLEs) were observed at any time during the challenge. Precautions were taken to correct changes in osmolality beyond 10 mOsm between solutions by adding sucrose as needed. Finally, in a subgroup of slices, the 1 s 100 Hz stimulus above described was repeated after the first 10 to 30 min of hypoglycemia in order to evaluate the susceptibility of the circuitry for generating after discharges or SLEs.

Pharmacology

5,5-Diphenylimidazolidine-2,4-dione (phenytoin), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (+)-5-methyl-10,11- dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801), and (2*R*)-amino-5-phosphonopentanoate (AP5), (6*R*)-6-[(5*S*)-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]furo[3,4-*e*][1,3]benzodioxol-8(6*H*)-one (Bicuculline), were acquired from Sigma-Aldrich. Stock solutions were prepared in ACSF or distilled water and diluted to the indicated concentrations for the different experiments.

Data analyses

Variables evaluated were the amplitude of field excitatory postsynaptic potentials (fEPSPs), and amplitude, frequency, and